BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 August 2003 (07.08.2003)

PCT

(10) International Publication Number
WO 03/063770 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US02/36098

(22) International Filing Date:
7 November 2002 (07.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/336,968 7 November 2001 (07.11.2001) US

(71) Applicant: CTL IMMUNOTHERAPIES CORP. [US/US]; 9340 DeSoto Avenue, Chatsworth, CA 91311 (US).

(72) Inventors: SIMARD, John, J.L.; Suite #7, 1684 Alberni Street, Vancouver, British Columbia Z6G1A6 (CA). DIAMOND, David, C.; 23135 Schoenborn Street, West Hills, CA 91304 (US). QUI, Zhiyong; 3232 Sawtelle Blvd., #107, Los Angeles, CA 90066 (US). LEI, Xiang-Dong; 6940 Newgate Road, West Hills, CA 91307 (US).

(74) Agent: HUNT, Dale, C.; Knobbe, Martens, Olson & Bear, LLP, 14th Floor, 2040 Main Street, Irvine, CA 92614 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS AND METHODS FOR THEIR DESIGN

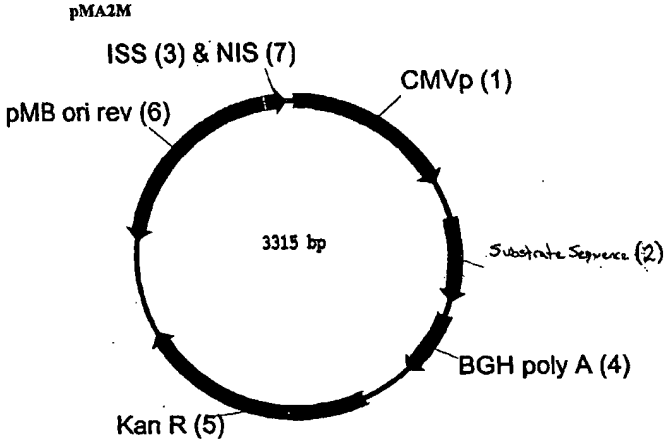


Figure Legend:

Code in Figure	Genetic Element	Region
~~1. CMVp~~	Cytomeglovirus Enhancer/Promotor —	63-637
2. Substrate Sequence	Substrate Sequence containing epitope.	696-983
3. ISS	Immunostimulatory Sequence	3220-3226
4. BGH poly A	Bovine Growth Hormone Polyadenylation Signal	1028-1045
5. Kan R	Kanamycin Resistance Gene	1431-2225
6. pMB ori rev	Bacterial pMB Origin of Replication	3165-2492
7. NIS	Nuclear Import sequence from Simian Virus 40-72bp repeat	3227-3304

(57) Abstract: The invention disclosed herein is directed to methods of identifying a polypeptide suitable for epitope liberation including, for example, the steps of identifying an epitope of interest; providing a substrate polypeptide sequence including the epitope, wherein the substrate polypeptide permits processing by a proteasome; contacting the substrate polypeptide with a composition including the proteasome, under conditions that support processing of the substrate polypeptide by the proteasome; and assaying for liberation of the epitope. The invention further relates to vectors including a housekeeping epitope expression cassette. The housekeeping epitope(s) can be derived from a target-associated antigen, and the housekeeping epitope can be liberatable, that is capable of liberation, from a translation product of the cassette by immunoproteasome processing. The invention also relates to a method of activating a T cell comprising contacting a substrate polypeptide with an APC and contacting the APC with a T cell.

WO 03/063770 A2

Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS AND METHODS FOR THEIR DESIGN

Background of the Invention

Field of the Invention

[0001] The invention disclosed herein is directed to methods for the design of epitope-encoding vectors for use in compositions, including for example, pharmaceutical compositions capable of inducing an immune response in a subject to whom the compositions are administered. The invention is further directed to the vectors themselves. The epitope(s) expressed using such vectors can stimulate a cellular immune response against a target cell displaying the epitope(s).

Description of the Related Art

[0002] The immune system can be categorized into two discrete effector arms. The first is innate immunity, which involves numerous cellular components and soluble factors that respond to all infectious challenges. The other is the adaptive immune response, which is customized to respond specifically to precise epitopes from infectious agents. The adaptive immune response is further broken down into two effector arms known as the humoral and cellular immune systems. The humoral arm is centered on the production of antibodies by B-lymphocytes while the cellular arm involves the killer cell activity of cytotoxic T Lymphocytes.

[0003] Cytotoxic T Lymphocytes (CTL) do not recognize epitopes on the infectious agents themselves. Rather, CTL detect fragments of antigens derived from infectious agents that are displayed on the surface of infected cells. As a result antigens are visible to CTL only after they have been processed by the infected cell and thus displayed on the surface of the cell.

[0004] The antigen processing and display system on the surface of cells has been well established. CTL recognize short peptide antigens, which are displayed on the surface in non-covalent association with class I major histocompatibility complex molecules (MHC). These class I peptides are in turn derived from the degradation of cytosolic proteins.

Summary of the Invention

[0005] Embodiments of the invention provide expression cassettes, for example, for use in vaccine vectors, which encode one or more embedded housekeeping epitopes, and methods for designing and testing such expression cassettes. Housekeeping epitopes can be liberated from the translation product of such cassettes through proteolytic processing by the immunoproteasome of professional antigen presenting cells (pAPC). In one embodiment of the invention, sequences flanking the housekeeping epitope(s) can be altered to promote cleavage by the immunoproteasome at the desired location(s). Housekeeping epitopes, their uses, and identification are described in U.S. Patent Application Nos. 09/560,465 and 09/561,074 entitled EPITOPE

SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS, and METHOD OF EPITOPE DISCOVERY, respectively; both of which were filed on April 28, 2000.

[0006] Examples of housekeeping epitopes are disclosed in provisional U.S. Patent Applications entitled EPITOPE SEQUENCES, Nos. 60/282,211, filed on April 6, 2001; 60/337,017, filed on November 7, 2001; 60/363210 filed 3/7/02; and 60/409,123, filed on September 5, 2002; and U.S. Application No. 10/117,937, filed on April 4, 2002, which is also entitled EPITOPE SEQUENCES.

[0007] In other embodiments of the invention, the housekeeping epitope(s) can be flanked by arbitrary sequences or by sequences incorporating residues known to be favored in immunoproteasome cleavage sites. As used herein the term "arbitrary sequences" refers to sequences chosen without reference to the native sequence context of the epitope, their ability to promote processing, or immunological function. In further embodiments of the invention multiple epitopes can be arrayed head-to-tail. These arrays can be made up entirely of housekeeping epitopes. Likewise, the arrays can include alternating housekeeping and immune epitopes. Alternatively, the arrays can include housekeeping epitopes flanked by immune epitopes, whether complete or distally truncated. Further, the arrays can be of any other similar arrangement. There is no restriction on placing a housekeeping epitope at the terminal positions of the array. The vectors can additionally contain authentic protein coding sequences or segments thereof containing epitope clusters as a source of immune epitopes. The term "authentic" refers to natural protein sequences.

[0008] Epitope clusters and their uses are described in U.S. Patent application Nos. 09/561,571 entitled EPITOPE CLUSTERS, filed on April 28, 2000; 10/005,905, entitled EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS, filed on November 7, 2001; and 10/026,066, filed on December 7, 2001, also entitled EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS.

[0009] Embodiments of the invention can encompass screening the constructs to determine whether the housekeeping epitope is liberated. In constructs containing multiple housekeeping epitopes, embodiments can include screening to determine which epitopes are liberated. In a preferred embodiment, a vector containing an embedded epitope can be used to immunize HLA transgenic mice and the resultant CTL can be tested for their ability to recognize target cells presenting the mature epitope. In another embodiment, target cells expressing immunoproteasome can be transformed with the vector. The target cell may express immunoproteasome either constitutively, because of treatment with interferon (IFN), or through genetic manipulation, for example. CTL that recognize the mature epitope can be tested for their ability to recognize these target cells. In yet another embodiment, the embedded epitope can be prepared as a synthetic peptide. The synthetic peptide then can be subjected to digestion by an

immunoproteasome preparation *in vitro* and the resultant fragments can be analyzed to determine the sites of cleavage. Such polypeptides, recombinant or synthetic, from which embedded epitopes can be successfully liberated, can also be incorporated into immunogenic compositions.

[0010] The invention disclosed herein relates to the identification of a polypeptide suitable for epitope liberation. One embodiment of the invention, relates to a method of identifying a polypeptide suitable for epitope liberation including, for example, the steps of identifying an epitope of interest; providing a substrate polypeptide sequence including the epitope, wherein the substrate polypeptide permits processing by a proteasome; contacting the substrate polypeptide with a composition including the proteasome, under conditions that support processing of the substrate polypeptide by the proteasome; and assaying for liberation of the epitope.

[0011] The epitope can be embedded in the substrate polypeptide, and in some aspects the substrate polypeptide can include more than one epitope, for example. Also, the epitope can be a housekeeping epitope.

[0012] In one aspect, the substrate polypeptide can be a synthetic peptide. Optionally, the substrate polypeptide can be included in a formulation promoting protein transfer. Alternatively, the substrate polypeptide can be a fusion protein. The fusion protein can further include a protein domain possessing protein transfer activity. Further, the contacting step can include immunization with the substrate polypeptide.

[0013] In another aspect, the substrate polypeptide can be encoded by a polynucleotide. The contacting step can include immunization with a vector including the polynucleotide, for example. The immunization can be carried out in an HLA-transgenic mouse or any other suitable animal, for example. Alternatively, the contacting step can include transforming a cell with a vector including the polynucleotide. In some embodiments the transformed cell can be a target cell that is targeted by CTL for purposes of assaying for proper liberation of epitope.

[0014] The proteasome processing can take place intracellularly, either *in vitro* or *in vivo*. Further, the proteasome processing can take place in a cell-free system.

[0015] The assaying step can include a technique selected from the group including, but not limited to, mass spectrometry, N-terminal pool sequencing, HPLC, and the like. Also, the assaying step can include a T cell target recognition assay. The T cell target recognition assay can be selected from the group including, but not limited to, a cytolytic activity assay, a chromium release assay, a cytokine assay, an ELISPOT assay, tetramer analysis, and the like.

[0016] In still another aspect, the amino acid sequence of the substrate polypeptide including the epitope can be arbitrary. Also, the substrate polypeptide in which the epitope is embedded can be derived from an authentic sequence of a target-associated antigen. Further, the substrate polypeptide in which the epitope is embedded can be conformed to a preferred immune proteasome cleavage site flanking sequence.

[0017] In another aspect, the substrate polypeptide can include an array of additional epitopes. Members of the array can be arranged head-to-tail, for example. The array can include more than one housekeeping epitope. The more than one housekeeping epitope can include copies of the same epitope. The array can include a housekeeping and an immune epitope, or alternating housekeeping and immune epitopes, for example. Also, the array can include a housekeeping epitope positioned between two immune epitopes in an epitope battery. The array can include multiple epitope batteries, so that there are two immune epitopes between each housekeeping epitope in the interior of the array. Optionally, at least one of the epitopes can be truncated distally to its junction with an adjacent epitope. The truncated epitopes can be immune epitopes, for example. The truncated epitopes can have lengths selected from the group including, but not limited to, 9, 8, 7, 6, 5, 4 amino acids, and the like.

[0018] In still another aspect, the substrate polypeptide can include an array of epitopes and epitope clusters. Members of the array can be arranged head-to-tail, for example.

[0019] In yet another aspect, the proteasome can be an immune proteasome.

[0020] Another embodiment of the disclosed invention relates to vectors including a housekeeping epitope expression cassette. The housekeeping epitope(s) can be derived from a target-associated antigen, and the housekeeping epitope can be liberatable, that is capable of liberation, from a translation product of the cassette by immunoproteasome processing.

[0021] In one aspect of the invention the expression cassette can encode an array of two or more epitopes or at least one epitope and at least one epitope cluster. The members of the array can be arranged head-to-tail, for example. Also, the members of the array can be arranged head-to-tail separated by spacing sequences, for example. Further, the array can include a plurality of housekeeping epitopes. The plurality of housekeeping epitopes can include more than one copy of the same epitope or single copies of distinct epitopes, for example. The array can include at least one housekeeping epitope and at least one immune epitope. Also, the array can include alternating housekeeping and immune epitopes. Further, the array includes a housekeeping epitope sandwiched between two immune epitopes so that there are two immune epitopes between each housekeeping epitope in the interior of the array. The immune epitopes can be truncated distally to their junction with the adjacent housekeeping epitope.

[0022] In another aspect, the expression cassette further encodes an authentic protein sequence, or segment thereof, including at least one immune epitope. Optionally, the segment can include at least one epitope cluster. The housekeeping epitope expression cassette and the authentic sequence including at least one immune epitope can be encoded in a single reading frame or transcribed as a single mRNA species, for example. Also, the housekeeping epitope expression cassette and the authentic sequence including at least one immune epitope may not be transcribed as a single mRNA species.

[0023] In yet another aspect, the vector can include a DNA molecule or an RNA molecule. The vector can encode, for example, SEQ ID NO. 4, SEQ ID NO. 17, SEQ ID NO. 20, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 33, and the like. Also, the vector can include SEQ ID NO. 9, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 30, SEQ ID NO. 34, and the like. Also, the vector can encode SEQ ID NO. 5 or SEQ ID NO. 18, for example.

[0024] In still another aspect, the target-associated antigen can be an antigen derived from or associated with a tumor or an intracellular parasite, and the intracellular parasite can be, for example, a virus, a bacterium, a protozoan, or the like.

[0025] Another embodiment of the invention relates to vectors including a housekeeping epitope identified according to any of the methods disclosed herein, claimed or otherwise. For example, embodiments can relate to vector encoding a substrate polypeptide that includes a housekeeping epitope by any of the methods described herein.

[0026] In one aspect, the housekeeping epitope can be liberated from the cassette translation product by immune proteasome processing

[0027] Another embodiment of the disclosed invention relates to methods of activating a T cell. The methods can include, for example, the steps of contacting a vector including a housekeeping epitope expression cassette with an APC. The housekeeping epitope can be derived from a target-associated antigen, for example, and the housekeeping epitope can be liberatable from a translation product of the cassette by immunoproteasome processing. The methods can further include contacting the APC with a T cell. The contacting of the vector with the APC can occur *in vitro* or *in vivo*.

[0028] Another embodiment of the disclosed invention relates to a substrate polypeptide including a housekeeping epitope wherein the housekeeping epitope can be liberated by immunoproteasome processing in a pAPC.

[0029] Another embodiment of the disclosed invention relates to a method of activating a T cell comprising contacting a substrate polypeptide including a housekeeping epitope with an APC wherein the housekeeping epitope can be liberated by immunoproteasome processing and contacting the APC with a T cell.

Brief Description of the Drawings

[0030] Figure 1. An illustrative drawing depicting pMA2M.

[0031] Figure 2. Assay results showing the % of specific lysis of ELAGIGILTV pulsed and unpulsed T2 target cells by mock immunized CTL.

[0032] Figure 3. Assay results showing the % of specific lysis of ELAGIGILTV pulsed and unpulsed T2 target cells by pVAXM3 immunized CTL.

[0033] Figure 4. Assay results showing the % of specific lysis of ELAGIGILTV pulsed and unpulsed T2 target cells by pVAXM2 immunized CTL.

[0034] Figure 5. Assay results showing the % of specific lysis of ELAGIGILTV pulsed and unpulsed T2 target cells by pVAXM1 immunized CTL.

[0035] Figure 6. Illustrates a sequence of SEQ ID NO. 22 from which the NY-ESO-$1_{157-165}$ epitope is liberated by immunoproteasomal processing.

[0036] Figure 7. Shows the differential processing by immunoproteasome and housekeeping proteasome of the SLLMWITQC epitope (SEQ ID NO. 12) in its native context where the cleavage following the C is more efficiently produced by housekeeping than immunoproteasome.

[0037] Figure 8. 8A: Shows the results of the human immunoproteasome digest of SEQ ID NO. 31. 8B: Shows the comparative results of mouse versus human immunoproteasome digestion of SEQ ID NO. 31.

[0038] Figure 9. Shows the differential processing of SSX-2_{31-68} by housekeeping and immunoproteasome.

Detailed Description of the Preferred Embodiment

Definitions

[0039] Unless otherwise clear from the context of the use of a term herein, the following listed terms shall generally have the indicated meanings for purposes of this description.

[0040] PROFESSIONAL ANTIGEN-PRESENTING CELL (pAPC) – a cell that possesses T cell costimulatory molecules and is able to induce a T cell response. Well characterized pAPCs include dendritic cells, B cells, and macrophages.

[0041] PERIPHERAL CELL – a cell that is not a pAPC.

[0042] HOUSEKEEPING PROTEASOME – a proteasome normally active in peripheral cells, and generally not present or not strongly active in pAPCs.

[0043] IMMUNOPROTEASOME – a proteasome normally active in pAPCs; the immunoproteasome is also active in some peripheral cells in infected tissues or following exposure to interferon.

[0044] EPITOPE – a molecule or substance capable of stimulating an immune response. In preferred embodiments, epitopes according to this definition include but are not necessarily limited to a polypeptide and a nucleic acid encoding a polypeptide, wherein the polypeptide is capable of stimulating an immune response. In other preferred embodiments, epitopes according to this definition include but are not necessarily limited to peptides presented on the surface of cells, the peptides being non-covalently bound to the binding cleft of class I MHC, such that they can interact with T cell receptors (TCR). Epitopes presented by class I MHC may be in immature or mature form. "Mature" refers to an MHC epitope in distinction to any precursor ("immature") that may include or consist essentially of a housekeeping epitope, but also includes other sequences in a primary translation product that are removed by processing, including without

limitation, alone or in any combination, proteasomal digestion, N-terminal trimming, or the action of exogenous enzymatic activities. Thus, a mature epitope may be provided embedded in a somewhat longer polypeptide, the immunological potential of which is due, at least in part, to the embedded epitope; or in its ultimate form that can bind in the MHC binding cleft to be recognized by TCR, respectively.

[0045] MHC EPITOPE – a polypeptide having a known or predicted binding affinity for a mammalian class I or class II major histocompatibility complex (MHC) molecule.

[0046] HOUSEKEEPING EPITOPE – In a preferred embodiment, a housekeeping epitope is defined as a polypeptide fragment that is an MHC epitope, and that is displayed on a cell in which housekeeping proteasomes are predominantly active. In another preferred embodiment, a housekeeping epitope is defined as a polypeptide containing a housekeeping epitope according to the foregoing definition, that is flanked by one to several additional amino acids. In another preferred embodiment, a housekeeping epitope is defined as a nucleic acid that encodes a housekeeping epitope according to the foregoing definitions. Exemplary housekeeping epitopes are provide in U.S. Application No. 10/117,937, filed on April 4, 2002; and U.S. Provisional Application Nos. 60/282,211, filed on April 6, 2001; 60/337,017, filed on November 7, 2001; 60/363210 filed 3/7/02; and 60/409,123, filed on September 5, 2002; all of which are entitled EPITOPE SEQUENCES.

[0047] IMMUNE EPITOPE – In a preferred embodiment, an immune epitope is defined as a polypeptide fragment that is an MHC epitope, and that is displayed on a cell in which immunoproteasomes are predominantly active. In another preferred embodiment, an immune epitope is defined as a polypeptide containing an immune epitope according to the foregoing definition, that is flanked by one to several additional amino acids. In another preferred embodiment, an immune epitope is defined as a polypeptide including an epitope cluster sequence, having at least two polypeptide sequences having a known or predicted affinity for a class I MHC. In yet another preferred embodiment, an immune epitope is defined as a nucleic acid that encodes an immune epitope according to any of the foregoing definitions.

[0048] TARGET CELL – a cell to be targeted by the vaccines and methods of the invention. Examples of target cells according to this definition include but are not necessarily limited to: a neoplastic cell and a cell harboring an intracellular parasite, such as, for example, a virus, a bacterium, or a protozoan. Target cells can also include cells that are targeted by CTL as a part of assays to determine or confirm proper epitope liberation and processing by a cell expressing immunoproteasome, to determine T cell specificity or immunogenicity for a desired epitope.. Such cells may be transfored to express the substrate or liberation sequence, or the cells can simply be pulsed with peptide/epitope.

[0049] TARGET-ASSOCIATED ANTIGEN (TAA) – a protein or polypeptide present in a target cell.

[0050] TUMOR-ASSOCIATED ANTIGENS (TuAA) – a TAA, wherein the target cell is a neoplastic cell.

[0051] HLA EPITOPE – a polypeptide having a known or predicted binding affinity for a human class I or class II HLA complex molecule.

[0052] ANTIBODY – a natural immunoglobulin (Ig), poly- or monoclonal, or any molecule composed in whole or in part of an Ig binding domain, whether derived biochemically or by use of recombinant DNA. Examples include *inter alia,* F(ab), single chain Fv, and Ig variable region-phage coat protein fusions.

[0053] ENCODE – an open-ended term such that a nucleic acid encoding a particular amino acid sequence can consist of codons specifying that (poly)peptide, but can also comprise additional sequences either translatable, or for the control of transcription, translation, or replication, or to facilitate manipulation of some host nucleic acid construct.

[0054] SUBSTANTIAL SIMILARITY – this term is used to refer to sequences that differ from a reference sequence in an inconsequential way as judged by examination of the sequence. Nucleic acid sequences encoding the same amino acid sequence are substantially similar despite differences in degenerate positions or modest differences in length or composition of any non-coding regions. Amino acid sequences differing only by conservative substitution or minor length variations are substantially similar. Additionally, amino acid sequences comprising housekeeping epitopes that differ in the number of N-terminal flanking residues, or immune epitopes and epitope clusters that differ in the number of flanking residues at either terminus, are substantially similar. Nucleic acids that encode substantially similar amino acid sequences are themselves also substantially similar.

[0055] FUNCTIONAL SIMILARITY – this term is used to refer to sequences that differ from a reference sequence in an inconsequential way as judged by examination of a biological or biochemical property, although the sequences may not be substantially similar. For example, two nucleic acids can be useful as hybridization probes for the same sequence but encode differing amino acid sequences. Two peptides that induce cross-reactive CTL responses are functionally similar even if they differ by non-conservative amino acid substitutions (and thus do not meet the substantial similarity definition). Pairs of antibodies, or TCRs, that recognize the same epitope can be functionally similar to each other despite whatever structural differences exist. In testing for functional similarity of immunogenicity one would generally immunize with the "altered" antigen and test the ability of the elicited response (Ab, CTL, cytokine production, etc.) to recognize the target antigen. Accordingly, two sequences may be designed to differ in certain

respects while retaining the same function. Such designed sequence variants are among the embodiments of the present invention.

[0056] EXPRESSION CASSETTE – a polynucleotide sequence encoding a polypeptide, operably linked to a promoter and other transcription and translation control elements, including but not limited to enhancers, termination codons, internal ribosome entry sites, and polyadenylation sites. The cassette can also include sequences that facilitate moving it from one host molecule to another.

[0057] EMBEDDED EPITOPE – an epitope contained within a longer polypeptide, also can include an epitope in which either the N- terminus or the C-terminus is embedded such that the epitope is not in an interior position.

[0058] MATURE EPITOPE – a peptide with no additional sequence beyond that present when the epitope is bound in the MHC peptide-binding cleft.

[0059] EPITOPE CLUSTER – a polypeptide, or a nucleic acid sequence encoding it, that is a segment of a native protein sequence comprising two or more known or predicted epitopes with binding affinity for a shared MHC restriction element, wherein the density of epitopes within the cluster is greater than the density of all known or predicted epitopes with binding affinity for the shared MHC restriction element within the complete protein sequence, and as disclosed in U.S. Patent Application No. 09/561,571 entitled EPITOPE CLUSTERS.

[0060] Substrate or liberation sequence– a designed or engineered sequence comprising or encoding a housekeeping epitope (according to the first of the definitions offered above) embedded in a larger sequence that provides a context allowing the housekeeping epitope to be liberated by immunoproteasomal processing, directly or in combination with N-terminal trimming or other processes.

[0061] Degradation of cytosolic proteins takes place via the ubiquitin-dependent multi-catalytic multi-subunit protease system known as the proteasome. The proteasome degrades cytosolic proteins generating fragments that can then be translocated from the cytosol into the endoplasmic reticulum (ER) for loading onto class I MHC. Such protein fragments shall be referred to as class I peptides. The peptide loaded MHC are subsequently transported to the cell surface where they can be detected by CTL.

[0062] The multi-catalytic activity of the proteasome is the result of its multi-subunit structure. Subunits are expressed from different genes and assembled post-translationally into the proteasome complex. A key feature of the proteasome is its bimodal activity, which enables it to exert its protease, or cleavage function, with two discrete kinds of cleavage patterns. This bimodal action of the proteasome is extremely fundamental to understanding how CTL are targeted to recognize peripheral cells in the body and how this targeting requires synchronization between the immune system and the targeted cells.

[0063] The housekeeping proteasome is constitutively active in all peripheral cells and tissues of the body. The first mode of operation for the housekeeping proteasome is to degrade cellular protein, recycling it into amino acids. Proteasome function is therefore a necessary activity for cell life. As a corollary to its housekeeping protease activity, however, class I peptides generated by the housekeeping proteasome are presented on all of the peripheral cells of the body.

[0064] The proteasome's second mode of function is highly exclusive and occurs specifically in pAPCs or as a consequence of a cellular response to interferons (IFNs). In its second mode of activity the proteasome incorporates unique subunits, which replace the catalytic subunits of the constitutive housekeeping proteasome. This "modified" proteasome has been called the immunoproteasome, owing to its expression in pAPC and as a consequence of induction by IFN in body cells.

[0065] APC define the repertoire of CTL that recirculate through the body and are potentially active as killer cells. CTL are activated by interacting with class I peptide presented on the surface of a pAPC. Activated CTL are induced to proliferate and caused to recirculate through the body in search of diseased cells. This is why the CTL response in the body is defined specifically by the class I peptides produced by the pAPC. It is important to remember that pAPCs express the immunoproteasome, and that as a consequence of the bimodal activity of the proteasome, the cleavage pattern of proteins (and the resultant class I peptides produced) are different from those in peripheral body cells which express housekeeping proteasome. The differential proteasome activity in pAPC and peripheral body cells, therefore, is important to consider during natural infection and with therapeutic CTL vaccination strategies.

[0066] All cells of the body are capable of producing IFN in the event that they are infected by a pathogen such as a virus. IFN production in turn results in the expression of the immunoproteasome in the infected cell. Viral antigens are thereby processed by the immunoproteasome of the infected cell and the consequent peptides are displayed with class I MHC on the cell surface. At the same time, pAPC are sequestering virus antigens and are processing class I peptides with their immunoproteasome activity, which is normal for the pAPC cell type. The CTL response in the body is being stimulated specifically by the class I peptides produced by the pAPC. Fortunately, the infected cell is also producing class I peptides from the immunoproteasome, rather than the normal housekeeping proteasome. Thus, virus-related class I peptides are being produced that enable detection by the ensuing CTL response. The CTL immune response is induced by pAPC, which normally produce different class I peptides compared to peripheral body cells, owing to different proteasome activity. Therefore, during infection there is epitope synchronization between the infected cell and the immune system.

[0067] This is not the case with tumors and chronic viruses, which block the interferon system. For tumors there is no infection in the tumor cell to induce the

immunoproteasome expression, and chronic virus infection either directly or indirectly blocks immunoproteasome expression. In both cases the diseased cell maintains its display of class I peptides derived from housekeeping proteasome activity and avoids effective surveillance by CTL.

[0068] In the case of therapeutic vaccination to eradicate tumors or chronic infections, the bimodal function of the proteasome and its differential activity in APC and peripheral cells of the body is significant. Upon vaccination with protein antigen, and before a CTL response can occur, the antigen must be acquired and processed into peptides that are subsequently presented on class I MHC on the pAPC surface. The activated CTL recirculate in search of cells with similar class I peptide on the surface. Cells with this peptide will be subjected to destruction by the cytolytic activity of the CTL. If the targeted diseased cell does not express the immunoproteasome, which is present in the pAPC, then the epitopes are not synchronized and CTL fail to find the desired peptide target on the surface of the diseased cell.

[0069] Preferably, therapeutic vaccine design takes into account the class I peptide that is actually present on the target tissue. That is, effective antigens used to stimulate CTL to attack diseased tissue are those that are naturally processed and presented on the surface of the diseased tissue. For tumors and chronic infection this generally means that the CTL epitopes are those that have been processed by the housekeeping proteasome. In order to generate an effective therapeutic vaccine, CTL epitopes are identified based on the knowledge that such epitopes are, in fact, produced by the housekeeping proteasome system. Once identified, these epitopes, embodied as peptides, can be used to successfully immunize or induce therapeutic CTL responses against housekeeping proteasome expressing target cells in the host.

[0070] However, in the case of DNA vaccines, there can be an additional consideration. The immunization with DNA requires that APCs take up the DNA and express the encoded proteins or peptides. It is possible to encode a discrete class I peptide on the DNA. By immunizing with this construct, APCs can be caused to express a housekeeping epitope, which is then displayed on class I MHC on the surface of the cell for stimulating an appropriate CTL response. Constructs for generation of proper termini of housekeeping epitopes have been described in U.S. Patent application No. 09/561,572 entitled EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS, filed on April 28, 2000.

[0071] Embodiments of the invention provide expression cassettes that encode one or more embedded housekeeping epitopes, and methods for designing and testing such expression cassettes. The expression cassettes and constructs can encode epitopes, including housekeeping epitopes, derived from antigens that are associated with targets. Housekeeping epitopes can be liberated from the translation product(s) of the cassettes. For example, in some embodiments of the invention, the housekeeping epitope(s) can be flanked by arbitrary sequences or by sequences incorporating residues known to be favored in immunoproteasome cleavage sites. In further

embodiments of the invention multiple epitopes can be arrayed head-to-tail. In some embodiments, these arrays can be made up entirely of housekeeping epitopes. Likewise, the arrays can include alternating housekeeping and immune epitopes. Alternatively, the arrays can include housekeeping epitopes flanked by immune epitopes, whether complete or distally truncated. In some preferred embodiments, each housekeeping epitope can be flanked on either side by an immune epitope, such that an array of such arrangements has two immune epitopes between each housekeeping epitope. Further, the arrays can be of any other similar arrangement. There is no restriction on placing a housekeeping epitope at the terminal positions of the array. The vectors can additionally contain authentic protein coding sequences or segments thereof containing epitope clusters as a source of immune epitopes.

[0072] Several disclosures make reference to polyepitopes or string-of-bead arrays. *See*, for example, WO0119408A1, March 22, 2001; WO9955730A2, November 4, 1999; WO0040261A2, July 13, 2000; WO9603144A1, February 8, 1996; EP1181314A1, February 27, 2002; WO0123577A3, April 5; US6074817, June 13, 2000; US5965381, October 12, 1999; WO9741440A1, November 6, 1997; US6130066, October 10, 2000; US6004777, December 21, 1999; US5990091, November 23, 1999; WO9840501A1, September 17, 1998; WO9840500A1, September 17, 1998; WO0118035A2, March 15, 2001; WO02068654A2, September 6, 2002; WO0189281A2, November 29, 2001; WO0158478A, August 16, 2001; EP1118860A1, July 25, 2001; WO0111040A1, February 15, 2001; WO0073438A1, December 7, 2000; WO0071158A1, November 30, 2000; WO0066727A1, November 9, 2000; WO0052451A1, September 8, 2000; WO0052157A1, September 8, 2000; WO0029008A2, May 25, 2000; WO0006723A1, February 10, 2000. Additional disclosures, include Palmowski MJ, et al - J Immunol 2002;168(9):4391-8; Fang ZY, et al - Virology 2001;291(2):272-84; Firat H, et al - J Gene Med 2002;4(1):38-45; Smith SG, et al - Clin Cancer Res 2001;7(12):4253-61; Vonderheide RH, et al - Clin Cancer Res 2001; 7(11):3343-8; Firat H, et al - Eur J Immunol 2001;31(10):3064-74; Le TT, et al - Vaccine 2001;19(32):4669-75; Fayolle C, et al - J Virol 2001;75(16):7330-8; Smith SG - Curr Opin Mol Ther 1999;1(1):10-5; Firat H, et al - Eur J Immunol 1999;29(10):3112-21; Mateo L, et al - J Immunol 1999;163(7):4058-63; Heemskerk MH, et al - Cell Immunol 1999;195(1):10-7; Woodberry T, et al - J Virol 1999;73(7):5320-5; Hanke T, et al - Vaccine 1998;16(4):426-35; Thomson SA, et al - J Immunol 1998;160(4):1717-23; Toes RE, et al - Proc Natl Acad Sci USA 1997;94(26):14660-5; Thomson SA, et al - J Immunol 1996;157(2):822-6; Thomson SA, et al - Proc Natl Acad Sci USA 1995;92(13):5845-9; Street MD, et al - Immunology 2002;106(4):526-36; Hirano K, et al - Histochem Cell Biol 2002;117(1):41-53; Ward SM, et al - Virus Genes 2001;23(1):97-104; Liu WJ, et al - Virology 2000;273(2):374-82; Gariglio P, et al - Arch Med Res 1998;29(4):279-84; Suhrbier A - Immunol Cell Biol 1997;75(4):402-8; Fomsgaard A, et al - Vaccine 1999;18(7-8):681-91; An LL, et al - J Virol 1997;71(3):2292-302; Whitton JL, et al - J

Virol 1993;67(1):348-52; Ripalti A, et al - J Clin Microbiol 1994;32(2):358-63; and Gilbert, S.C., et al., Nat. Biotech. 15:1280-1284, 1997.

[0073] One important feature that the disclosures in the preceding paragraph all share is their lack of appreciation for the desirability of regenerating housekeeping epitopes when the construct is expressed in a pAPC. This understanding was not apparent until the present invention. Embodiments of the invention include sequences, that when processed by an immune proteasome, liberate or generate a housekeeping epitope. Embodiments of the invention also can liberate or generate such epitopes in immunogenically effective amounts. Accordingly, while the preceding references contain disclosures relating to polyepitope arrays, none is enabling of the technology necessary to provide or select a polyepitope capable of liberating a housekeeping epitope by action of an immunoproteasome in a pAPC. In contrast, embodiments of the instant invention are based upon a recognition of the desirability of achieving this result. Accordingly, embodiments of the instant invention include any nucleic acid construct that encodes a polypeptide containing at least one housekeeping epitope provided in a context that promotes its generation via immunoproteasomal activity, whether the housekeeping epitope is embedded in a string-of-beads array or some other arrangement. Some embodiments of the invention include uses of one or more of the nucleic acid constructs or their products that are specifically disclosed in any one or more of the above-listed references. Such uses include, for example, screening a polyepitope for proper liberation context of a housekeeping epitope and/or an immune epitope, designing an effective immunogen capable of causing presentation of a housekeeping epitope and/or an immune epitope on a pAPC, immunizing a patient, and the like. Alternative embodiments include use of only a subset of such nucleic acid constructs or a single such construct, while specifically excluding one or more other such constructs, for any of the purposes disclosed herein. Some preferred embodiments employ these and/or other nucleic acid sequences encoding polyepitope arrays alone or in combination. For example, some embodiments exclude use of polyepitope arrays from one or more of the above-mentioned references. Other embodiments may exclude any combination or all of the polyepitope arrays from the above-mentioned references collectively. Some embodiments include viral and/or bacterial vectors encoding polyepitope arrays, while other embodiments specifically exclude such vectors. Such vectors can encode carrier proteins that may have some immunostimulatory effect. Some embodiments include such vectors with such immunostimulatory/immunopotentiating effects, as opposed to immunogenic effects, while in other embodiments such vectors may be included. Further, in some instances viral and bacterial vectors encode the desired epitope as a part of substantially complete proteins which are not associated with the target cell. Such vectors and products are included in some embodiments, while excluded from others. Some embodiments relate to repeated administration of vectors. In some of those embodiments, nonviral and nonbacterial vectors are included. Likewise, some embodiments

include arrays that contain extra amino acids between epitopes, for example anywhere from 1-6 amino acids, or more, in some embodiments, while other embodiments specifically exclude such arrays.

[0074] Embodiments of the present invention also include methods, uses, therapies, and compositions directed to various types of targets. Such targets can include, for example, neoplastic cells such as those listed below, for example; and cells infected with any virus, bacterium, protozoan, fungus, or other agents, examples of which are listed below, in Tables 1-5, or which are disclosed in any of the references listed above. Alternative embodiments include the use of only a subset of such neoplastic cells and infected cells listed below, in Tables 1-5, or in any of the references disclosed herein, or a single one of the neoplastic cells or infected cells, while specifically excluding one or more other such neoplastic cells or infected cells, for any of the purposes disclosed herein. The following are examples of neoplastic cells that can be targeted: human sarcomas and carcinomas, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g., acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, hepatocellular cancer, brain cancer, stomach cancer, liver cancer, and the like. Examples of infectious agents that infect the target cells can include the following: adenovirus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 6, varicella-zoster virus, hepatitis B virus, hepatitis D virus, papilloma virus, parvovirus B19, polyomavirus BK, polyomavirus JC, hepatitis C virus, measles virus, rubella virus, human immunodeficiency virus (HIV), human T cell leukemia virus I, human T cell leukemia virus II, *Chlamydia*, *Listeria*, *Salmonella*, *Legionella*, *Brucella*, *Coxiella*, *Rickettsia*, *Mycobacterium*, *Leishmania*, *Trypanasoma*, *Toxoplasma*, *Plasmodium*, and the like. Exemplary infectious agents and neoplastic cells are also included in Tables 1-5 below.

[0075] Furthermore the targets can include neoplastic cells described in or cells infected by agents that are described in any of the following references: Jäger, E. et al., "Granulocyte-macrophage-colony-stimulating factor enhances immune responses to melanoma-associated peptides *in vivo*," *Int. J Cancer*, 67:54-62 (1996); Kündig, T.M., Althage, A., Hengartner, H. & Zinkernagel, R.M., "A skin test to assess CD8+ cytotoxic T cell activity," *Proc. Natl. Acad Sci. USA*, 89:7757-76 (1992); Bachmann, M.F. & Kundig, T.M., "*In vitro* vs. *in vivo* assays for the assessment of T- and B-cell function," *Curr. Opin. Immunol.*, 6:320-326 (1994); Kundig et al., "On the role of antigen in maintaining cytotoxic T cell memory," *Proceedings of the National Academy of Sciences of the United States of America,* 93:9716-23 (1996); Steinmann, R.M., "The dendritic cells system and its role in irnmunogenicity," *Annual Review of Immunology* 9:271-96 (1991); Inaba, K. et al., "Identification of proliferating dendritic cell precursors in mouse blood," *Journal of Experimental Medicine,* 175:1157-67 (1992); Young, J.W. & Inaba, K., "Dendritic cells as adjuvants for class I major histocompatibility complex-restricted anti-tumor immunity," *Journal of Experimental Medicine,* 183:7-11 (1996); Kuby, Janis, *Immunology,* Second Edition, Chapter 15, W.H. Freeman and Company (1991); Austenst, E., Stahl, T., and de Gruyter, Walter, *Insulin Pump Therapy*, Chapter 3, Berlin, New York (1990); Remington, *The Science and Practice of Pharmacy*, Nineteenth Edition, Chapters 86-88; Cleland, Jeffery L. and Langer, Robert (Editor), "Formulation and delivery of proteins and peptides," *American Chemical Society* (ACS Symposium Series, No. 567) (1994); Dickinson, Becton, which is fixed using Tegadenn transparent dressing Tegaderm™ 1624, 3M, St. Paul, MN 55144, USA; Santus, Giancarlo and Baker, Richard, "Osmotic drug delivery: A review of the patent literature," *Journal of Controlled Release*, 35:1-21 (1995); Rammensee, U.S. Patent No. 5,747,269, issued May 05, 1998; Magruder, U.S. Patent No. 5,059,423, issued October 22, 1991; Sandbrook, U.S. Patent No. 4,552,651, issued November 25, 1985; Eckenhoff et al., U.S. Patent No. 3,987,790, issued October 26, 1976; Theeuwes, U.S. Patent No. 4,455,145, issued June 19, 1984; Roth et al. U.S. Patent No. 4,929,233, issued May 29 1990; van der Bruggen et al., U.S. Patent No. 5,554,506, issued September 10, 1996; Pfreundschuh, U.S. Patent No. 5,698,396, issued December 16, 1997; Magruder, U.S. Patent No. 5,110,596, issued May 5, 1992; Eckenhoff, U.S. Patent No. 4,619,652, issued October 28, 1986; Higuchi et al., U.S. Patent No. 3,995,631, issued December 07, 1976; Maruyama, U.S. Patent No. 5,017,381, issued May 21, 1991; Eckenhoff, U.S. Patent No. 4,963,141, issued October 16, 1990; van der Bruggen et al., U.S. Patent No. 5,558,995, issued September 24, 1996; Stolzenberg et al. U.S. Patent No. 3,604,417, issued September 14, 1971; Wong et al., U.S. Patent No. 5,110,597, issued May 05, 1992; Eckenhoff, U.S. Patent No. 4,753,651, issued June 28, 1988; Theeuwes, U.S. Patent No. 4,203,440, issued May 20, 1980; Wong et al. U.S. Patent No. 5,023,088, issued June 11, 1991; Wong et al., U.S. Patent No. 4,976,966, issued December 11, 1990; Van den Eynde et al., U.S. Patent No. 5,648,226, issued

July 15, 1997; Baker et al., U.S. Patent No. 4,838,862, issued June 13, 1989; Magruder, U.S. Patent No. 5,135,523, issued August 04, 1992; Higuchi et al., U.S. Patent No. 3,732,865, issued May 15, 1975, ; Theeuwes, U.S. Patent No. 4,286,067, issued August, 25 1981; Theeuwes et al., U.S. Patent No. 5,030,216, issued July 09, 1991; Boon et al., U.S. Patent No. 5,405,940, issued April 11, 1995; Faste, U.S. Patent No. 4,898,582, issued February 06, 1990; Eckenhoff, U.S. Patent No. 5,137,727, issued August 11, 1992; Higuchi et al., U.S. Patent No. 3,760,804, issued September 25, 1973; Eckenhoff et al., U.S. Patent No. 4,300,558, issued November 12, 1981; Magruder et al., U.S. Patent No. 5,034,229, issued July 23, 1991; Boon et al., U.S. Patent No. 5,487,974, issued January 30, 1996; Kam et al., U.S. Patent No. 5,135,498, issued August 04, 1992; Magruder et al., U.S. Patent No. 5,174,999, issued December 29, 1992; Higuchi, U.S. Patent No. 3,760,805, September 25, 1973; Michaels, U.S. Patent No. 4,304,232, issued December 08, 1981; Magruder et al., U.S. Patent No. 5,037,420, issued October 15, 1991; Wolfel et al., U.S. Patent No. 5,530,096, issued June 25, 1996; Athadye et al., U.S. Patent No. 5,169,390, issued December 08, 1992; Balaban et al., U.S. Patent No. 5,209,746, issued May 11, 1993; Higuchi, U.S. Patent No. 3,929,132, issued December 30, 1975; Michaels, U.S. Patent No. 4,340,054, issued July 20, 1982; Magruder et al., U.S. Patent No. 5,057,318, issued October 15, 1991; Wolfel et al., U.S. Patent No. 5,519,117, issued May 21, 1996; Athadye et al., U.S. Patent No. 5,257,987, issued November 02, 1993; Linkwitz et al., U.S. Patent No. 5,221,278, issued June 22, 1993; Nakano et al., U.S. Patent No. 3,995,632, issued December 07, 1976; Michaels, U.S. Patent No. 4,367,741, issued January 11, 1983; Eckenhoff, U.S. Patent No. 4,865,598, issued September 12, 1989; Lethe et al., U.S. Patent No. 5,774,316, issued April 28, 1998; Eckenhoff, U.S. Patent No. 4,340,048, issued July 20, 1982; Wong, U.S. Patent No. 5,223,265, issued June 29, 1993; Higuchi et al., U.S. Patent No. 4,034,756, issued July 12, 1977; Michaels, U.S. Patent No. 4,450,198, issued May 22, 1984; Eckenhoff et al., U.S. Patent No. 4,865,845, issued September 12, 1989; Melief et. al., U.S. Patent No. 5,554,724, issued September 10, 1996; Eckenhoff et al., U.S. Patent No. 4,474,575, issued October 02, 1984; Theeuwes, U.S. Patent No. 3,760,984, issued September 25, 1983; Eckenhoff, U.S. Patent No. 4,350,271, issued September 21, 1982; Eckenhoff et al., U.S. Patent No. 4,855,141, issued August 08, 1989; Zingerman, U.S. Patent No. 4,872,873, issued October 10, 1989; Townsend et al., U.S. Patent No. 5,585,461, issued December 17, 1996; Carulli, J.P. et al., *J. Cellular Biochem Suppl.*, 30/31:286-96 (1998); Türeci, Ö., Sahin, U., and Pfreundschuh, M., "Serological analysis of human tumor antigens: molecular definition and implications," *Molecular Medicine Today*, 3:342 (1997); Rammensee et al., *MHC Ligands and Peptide Motifs*, Landes Bioscience Austin, TX, 224-27, (1997); Parker et al., "Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains," *J. Immunol.* 152:163-175; Kido & Ohshita, *Anal. Biochem.*, 230:41-47 (1995); Yamada et al., *J. Biochem.* (Tokyo), 95:1155-60 (1984); Kawashima et al., *Kidney Int.*, 54:275-8 (1998);

Nakabayshi & Ikezawa, *Biochem. Int.* 16:1119-25 (1988); Kanaseki & Ohkuma, *J. Biochem.* (Tokyo), 110:541-7 (1991); Wattiaux et al., *J. Cell Biol.*, 78:349-68 (1978); Lisman et al., *Biochem. J.*, 178:79-87 (1979); Dean, B., *Arch. Biochem. Biophys.*, 227:154-63 (1983); Overdijk et al., *Adv. Exp. Med. Biol.*, 101:601-10 (1978); Stromhaug et al., *Biochem. J.*, 335:217-24 (1998); Escola et al., *J. Biol. Chem.*, 271:27360-05 (1996); Hammond et al., *Am. J. Physiol.*, 267:F516-27 (1994); Williams & Smith, *Arch. Biochem. Biophys.*, 305:298-306 (1993); Marsh, M., *Methods Cell Biol.*, 31:319-34 (1989); Schmid & Mellman, *Prog. Clin. Biol. Res.*, 270:35-49 (1988); Falk, K. et al., *Nature,* 351:290, (1991); Ausubel et al., *Short Protocols in Molecular Biology*, Third Edition, Unit 11.2 (1997); hypertext transfer protocol address 134.2.96.221/scripts/hlaserver.dll/EpPredict.htm; Levy, Morel, S. et al., *Immunity* 12:107-117 (2000); Seipelt et al., *Virus Research*, 62:159-68, 1999; Storkus et al., U.S. Patent No. 5,989,565, issued November 23, 1999; Morton, U.S. Patent No. 5,993,828, issued November 30, 1999; *Virus Research* 62:159-168, (1999); Simard et al., U.S. Patent Application No. 10/026066, filed December 07, 2001; Simard et al., U.S. Patent Application No. 09/561571, filed April 28, 2000; Simard et al., U.S. Patent Application No. 09/561572, filed April 28, 2000; Miura et al., WO 99/01283, January 14, 1999; Simard et al., U.S. Patent Application No. 09/561074, filed April 28, 2000; Simard et al., U.S. Patent Application No. 10/225568, filed August 20, 2002; Simard et al., U.S. Patent Application No. 10/005905, filed November 07, 2001; Simard et al., U.S. Patent Application No. 09/561074, filed April 28, 2000.

[0076] Additional embodiments of the invention include methods, uses, therapies, and compositions relating to a particular antigen, whether the antigen is derived from, for example, a target cell or an infective agent, such as those mentioned above. Some preferred embodiments employ the antigens listed herein, in Tables 1-5, or in the list below, alone, as subsets, or in any combination. For example, some embodiments exclude use of one or more of those antigens. Other embodiments may exclude any combination or all of those antigens. Several examples of such antigens include MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, CEA, RAGE, NY-ESO, SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, p16, as well as any of those set forth in the above mentioned references. Other antigens are included in Tables 1-4 below.

[0077] Further embodiments include methods, uses, compositions, and therapies relating to epitopes, including, for example those epitopes listed in Tables 1-5. These epitopes can be useful to flank housekeeping epitopes in screening vectors, for example. Some embodiments

include one or more epitopes from Tables 1-5, while other embodiments specifically exclude one or more of such epitopes or combinations thereof.

Table 1

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule
Adenovirus 3	E3 9Kd	30-38	LIVIGILIL	HLA-A*0201
			(SEQ. ID NO.:44)	
Adenovirus 5	EIA	234-243	SGPSNTPPEI	H2-Db
			(SEQ. ID NO.:45)	
Adenovirus 5	EIB	192-200	VNIRNCCYI	H2-Db
			(SEQ. ID NO.:46)	
Adenovirus 5	EIA	234-243	SGPSNIPPEI (T>I)	H2-Db
			(SEQ. ID NO.:47)	
CSFV	NS polyprotein	2276-2284	ENALLVALF	SLA,haplotype d/d
			(SEQ. ID NO.:48)	
Dengue virus 4	NS3	500-508	TPEGIIPTL	HLA-B*3501
			(SEQ. ID NO.:49)	
EBV	LMP-2	426-434	CLGGLLTMV	HLA-A*0201
			(SEQ. ID NO.:50)	
EBV	EBNA-1	480-484	NIAEGLRAL	HLA-A*0201
			(SEQ. ID NO.:51)	
EBV	EBNA-1	519-527	NLRRGTALA	HLA-A*0201
			(SEQ. ID NO.:52)	
EBV	EBNA-1	525-533	ALAIPQCRL	HLA-A*0201
			(SEQ. ID NO.:53)	
EBV	EBNA-1	575-582	VLKDAIKDL	HLA-A*0201
			(SEQ. ID NO.:54)	
EBV	EBNA-1	562-570	FMVFLQTHI	HLA-A*0201
			(SEQ. ID NO.:55)	
EBV	EBNA-2	15-23	HLIVDTDSL	HLA-A*0201
			(SEQ. ID NO.:56)	
EBV	EBNA-2	22-30	SLGNPSLSV	HLA-A*0201
			(SEQ. ID NO.:57)	
EBV	EBNA-2	126-134	PLASAMRML	HLA-A*0201
			(SEQ. ID NO.:58)	
EBV	EBNA-2	132-140	RMLWMANYI	HLA-A*0201
			(SEQ. ID NO.:59)	
EBV	EBNA-2	133-141	MLWMANYIV	HLA-A*0201
			(SEQ. ID NO.:60)	
EBV	EBNA-2	151-159	ILPQGPQTA	HLA-A*0201
			(SEQ. ID NO.:61)	
EBV	EBNA-2	171-179	PLRPTAPTI	HLA-A*0201
			(SEQ. ID NO.:62)	
EBV	EBNA-2	205-213	PLPPATLTV	HLA-A*0201
			(SEQ. ID NO.:63)	
EBV	EBNA-2	246-254	RMHLPVLHV	HLA-A*0201
			(SEQ. ID NO.:64)	
EBV	EBNA-2	287-295	PMPLPPSQL	HLA-A*0201
			(SEQ. ID NO.:65)	

EBV	EBNA-2	294-302	QLPPPAAPA	HLA-A*0201
			(SEQ. ID NO.:66)	
EBV	EBNA-2	381-389	SMPELSPVL	HLA-A*0201
			(SEQ. ID NO.:67)	
EBV	EBNA-2	453-461	DLDESWDYI	HLA-A*0201
			(SEQ. ID NO.:68)	
EBV	BZLF1	43-51	PLPCVLWPV	HLA-A*0201
			(SEQ. ID NO.:69)	
EBV	BZLF1	167-175	SLEECDSEL	HLA-A*0201
			(SEQ. ID NO.:70)	
EBV	BZLF1	176-184	EIKRYKNRV	HLA-A*0201
			(SEQ. ID NO.:71)	
EBV	BZLF1	195-203	QLLQHYREV	HLA-A*0201
			(SEQ. ID NO.:72)	
EBV	BZLF1	196-204	LLQHYREVA	HLA-A*0201
			(SEQ. ID NO.:73)	
EBV	BZLF1	217-225	LLKQMCPSL	HLA-A*0201
			(SEQ. ID NO.:74)	
EBV	BZLF1	229-237	SIIPRTPDV	HLA-A*0201
			(SEQ. ID NO.:75)	
EBV	EBNA-6	284-293	LLDFVRFMGV	HLA-A*0201
			(SEQ. ID NO.:76)	
EBV	EBNA-3	464-472	SVRDRLARL	HLA-A*0203
			(SEQ. ID NO.:77)	
EBV	EBNA-4	416-424	IVTDFSVIK	HLA-A*1101
			(SEQ. ID NO.:78)	
EBV	EBNA-4	399-408	AVFDRKSDAK	HLA-A*0201
			(SEQ. ID NO.:79)	
EBV	EBNA-3	246-253	RYSIFFDY	HLA-A24
			(SEQ. ID NO.:80)	
EBV	EBNA-6	881-889	QPRAPIRPI	HLA-B7
			(SEQ. ID NO.:81)	
EBV	EBNA-3	379-387	RPPIFIRRI.	HLA-B7
			(SEQ. ID NO.:82)	
EBV	EBNA-1	426-434	EPDVPPGAI	HLA-B7
			(SEQ. ID NO.:83)	
EBV	EBNA-1	228-236	IPQCRLTPL	HLA-B7
			(SEQ. ID NO.:84)	
EBV	EBNA-1	546-554	GPGPQPGPL	HLA-B7
			(SEQ. ID NO.:85)	
EBV	EBNA-1	550-558	QPGPLRESI	HLA-B7
			(SEQ. ID NO.:86)	
EBV	EBNA-1	72-80	R.PQKRPSCI	HLA-B7
			(SEQ. ID NO.:87)	
EBV	EBNA-2	224-232	PPTPLLTVL	HLA-B7
			(SEQ. ID NO.:88)	
EBV	EBNA-2	241-249	TPSPPRMHL	HLA-B7
			(SEQ. ID NO.:89)	
EBV	EBNA-2	244-252	PPRMHLPVL	HLA-B7
			(SEQ. ID NO.:90)	
EBV	EBNA-2	254-262	VPDQSMHPL	HLA-B7

			(SEQ. ID NO.:91)	
EBV	EBNA-2	446-454	PPSIDPADL	HLA-B7
			(SEQ. ID NO.:92)	
EBV	BZLFI	44-52	LPCVLWPVL	HLA-B7
			(SEQ. ID NO.:93)	
EBV	BZLF1	222-231	CPSLDVDSII	HLA-B7
			(SEQ. ID NO.:94)	
EBV	BZLFI	234-242	TPDVLHEDL	HLA-B7
			(SEQ. ID NO.:95)	
EBV	EBNA-3	339-347	FLRGRAYGL	HLA-B8
			(SEQ. ID NO.:96)	
EBV	EBNA-3	26-34	QAKWRLQTL	HLA-B8
			(SEQ. ID NO.:97)	
EBV	EBNA-3	325-333	AYPLHEQHG	HLA-B8
			(SEQ. ID NO.:98)	
EBV	EBNA-3	158-166	YlKSFVSDA	HLA-B8
			(SEQ. ID NO.:99)	
EBV	LMP-2	236-244	RRRWRRLTV	HLA-B*2704
			(SEQ. ID NO.:100)	
EBV	EBNA-6	258-266	RRIYDLIEL	HLA-B*2705
			(SEQ. ID NO.:101)	
EBV	EBNA-3	458-466	YPLHEQHGM	HLA-B*3501
			(SEQ. ID NO.:102)	
EBV	EBNA-3	458-466	YPLHEQHGM	HLA-B*3503
			(SEQ. ID NO.:103)	
HCV	NS3	389-397	HSKKKCDEL	HLA-B8
			(SEQ. ID NO.:104)	
HCV	env E	44-51	ASRCWVAM	HLA-B*3501
			(SEQ. ID NO.:105)	
HCV	core protein	27-35	GQIVGGVYL	HLA-B*40012
			(SEQ. ID NO.:106)	
HCV	NSI	77-85	PPLTDFDQGW	HLA-B*5301
			(SEQ. ID NO.:107)	
HCV	core protein	18-27	LMGYIPLVGA	H2-Dd
			(SEQ. ID NO.:108)	
HCV	core protein	16-25	ADLMGYIPLV	H2-Dd
			(SEQ. ID NO.:109)	
HCV	NS5	409-424	MSYSWTGALVTPCAEE	H2-Dd
			(SEQ. ID NO.:110)	
HCV	NS1	205-213	KHPDATYSR	Papa-A06
			(SEQ. ID NO.:111)	
HCV-1	NS3	400-409	KLVALGINAV	HLA-A*0201
			(SEQ. ID NO.:112)	
HCV-1	NS3	440-448	GDFDSVIDC	Patr-B16
			(SEQ. ID NO.:113)	
HCV-1	env E	118-126	GNASRCWVA	Patr-BI6
			(SEQ. ID NO.:114)	
HCV-1	NSI	159-167	TRPPLGNWF	Patr-B13
			(SEQ. ID NO.:115)	
HCV-1	NS3	351-359	VPHPNIEEV	Patr-B13
			(SEQ. ID NO.:116)	

HCV-1	NS3	438-446	YTGDFDSVI	Patr-B01
			(SEQ. ID NO.:117)	
HCV-1	NS4	328-335	SWAIKWEY	Patr-Al 1
			(SEQ. ID NO.:118)	
HCV-1	NSI	205-213	KHPDATYSR	Patr-A04
			(SEQ. ID NO.:119)	
HCV-1	NS3	440-448	GDFDSVIDC	Patr-A04
			(SEQ. ID NO.:120)	
HIV	gp4l	583-591	RYLKDQQLL	HLA_A24
			(SEQ. ID NO.:121)	
HIV	gagp24	267-275	IVGLNKIVR	HLA-A*3302
			(SEQ. ID NO.:122)	
HIV	gagp24	262-270	EIYKRWIIL	HLA-B8
			(SEQ. ID NO.:123)	
HIV	gagp24	261-269	GEIYKRWII	HLA-B8
			(SEQ. ID NO.:124)	
HIV	gagp17	93-101	EIKDTKEAL	HLA-B8
			(SEQ. ID NO.:125)	
HIV	gp4l	586-593	YLKDQQLL	HLA-B8
			(SEQ. ID NO.:126)	
HIV	gagp24	267-277	ILGLNKIVRMY	HLA-B* 1501
			(SEQ. ID NO.:127)	
HIV	gp4l	584-592	ERYLKDQQL	HLA-B14
			(SEQ. ID NO.:128)	
HIV	nef	115-125	YHTQGYFPQWQ	HLA-B17
			(SEQ. ID NO.:129)	
HIV	nef	117-128	TQGYFPQWQNYT	HLA-B17
			(SEQ. ID NO.:130)	
HIV	gpl20	314-322	GRAFVTIGK	HLA-B*2705
			(SEQ. ID NO.:131)	
HIV	gagp24	263-271	KRWIILGLN	HLA-B*2702
			(SEQ. ID NO.:132)	
HIV	nef	72-82	QVPLRPMTYK	HLA-B*3501
			(SEQ. ID NO.:133)	
HIV	nef	117-125	TQGYFPQWQ	HLA-B*3701
			(SEQ. ID NO.:134)	
HIV	gagp24	143-151	HQAISPRTI,	HLA-Cw*0301
			(SEQ. ID NO.:135)	
HIV	gagp24	140-151	QMVHQAISPRTL	HLA-Cw*0301
			(SEQ. ID NO.:136)	
HIV	gpl20	431-440	MYAPPIGGQI	H2-Kd
			(SEQ. ID NO.:137)	
HIV	gpl60	318-327	RGPGRAFVTI	H2-Dd
			(SEQ. ID NO.:138)	
HIV	gp120	17-29	MPGRAFVTI	H2-Ld
			(SEQ. ID NO.:139)	
HIV-1	RT	476-484	ILKEPVHGV	HLA-A*0201
			(SEQ. ID NO.:140)	
HIV-1	nef	190-198	AFHHVAREL	HLA-A*0201
			(SEQ. ID NO.:141)	
HIV-1	gpI60	120-128	KLTPLCVTL	HLA-A*0201

			(SEQ. ID NO.:142)	
HIV-1	gp160	814-823	SLLNATDIAV	HLA-A*0201
			(SEQ. ID NO.:143)	
HIV-1	RT	179-187	VIYQYMDDL	HLA-A*0201
			(SEQ. ID NO.:144)	
HIV-1	gagp 17	77-85	SLYNTVATL	HLA-A*0201
			(SEQ. ID NO.:145)	
HIV-1	gp160	315-329	RGPGRAFVTI	HLA-A*0201
			(SEQ. ID NO.:146)	
HIV-1	gp41	768-778	RLRDLLLIVTR	HLA-A3
			(SEQ. ID NO.:147)	
HIV-1	nef	73-82	QVPLRPMTYK	HLA-A3
			(SEQ. ID NO.:148)	
HIV-1	gp120	36-45	TVYYGVPVWK	HLA-A3
			(SEQ. ID NO.:149)	
HIV-1	gagp17	20-29	RLRPGGKKK	HLA-A3
			(SEQ. ID NO.:150)	
HIV-1	gp120	38-46	VYYGVPVWK	HLA-A3
			(SEQ. ID NO.:151)	
HIV-1	nef	74-82	VPLRPMTYK	HLA-a*1101
			(SEQ. ID NO.:152)	
HIV-1	gagp24	325-333	AIFQSSMTK	HLA-A*1101
			(SEQ. ID NO.:153)	
HIV-1	nef	73-82	QVPLRPMTYK	HLA-A*1101
			(SEQ. ID NO.:154)	
HIV-1	nef	83-94	AAVDLSHFLKEK	HLA-A*1101
			(SEQ. ID NO.:155)	
HIV-1	gagp24	349-359	ACQGVGGPGGHK	HLA-A*1101
			(SEQ. ID NO.:156)	
HIV-1	gagp24	203-212	ETINEEAAEW	HLA-A25
			(SEQ. ID NO.:157)	
HIV-1	nef	128-137	TPGPGVRYPL	HLA-B7
			(SEQ. ID NO.:158)	
HIV-1	gagp 17	24-31	GGKKKYKL	HLA-B8
			(SEQ. ID NO.:159)	
HIV-1	gp120	2-10	RVKEKYQHL	HLA-B8
			(SEQ. ID NO.:160)	
HIV-1	gagp24	298-306	DRFYKTLRA	HLA-B 14
			(SEQ. ID NO.:161)	
HIV-1	NEF	132-147	GVRYPLTFGWCYKLVP	HLA-B18
			(SEQ. ID NO.:162)	
HIV-1	gagp24	265-24	KRWIILGLNK	HLA-B*2705
			(SEQ. ID NO.:163)	
HIV-1	nef	190-198	AFHHVAREL	HLA-B*5201
			(SEQ. ID NO.:164)	
EBV	EBNA-6	335-343	KEHVIQNAF	HLA-B44
			(SEQ. ID NO.:165)	
EBV	EBNA-6	130-139	EENLLDFVRF	HLA-B*4403
			(SEQ. ID NO.:166)	
EBV	EBNA-2	42-51	DTPLIPLTIF	HLA-B51
			(SEQ. ID NO.:167)	

EBV	EBNA-6	213-222	QNGALAINTF	HLA-1362
			(SEQ. ID NO.:168)	
EBV	EBNA-3	603-611	RLRAEAGVK	HLA-A3
			(SEQ. ID NO.:169)	
HBV	sAg	348-357	GLSPTVWLSV	HLA-A*0201
			(SEQ. ID NO.:170)	
HBV	SAg	335-343	WLSLLVPFV	HLA-A*0201
			(SEQ. ID NO.:171)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*0201
			(SEQ. ID NO.:172)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*0202
			(SEQ. ID NO.:173)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*0205
			(SEQ. ID NO.:174)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*0206
			(SEQ. ID NO.:175)	
HBV	pol	575-583	FLLSLGIHL	HLA-A*0201
			(SEQ. ID NO.:176)	
HBV	pol	816-824	SLYADSPSV	HLA-A*0201
			(SEQ. ID NO.:177)	
HBV	pol	455-463	GLSRYVARL	HLA-A*0201
			(SEQ. ID NO.:178)	
HBV	env	338-347	LLVPFVQWFV	HLA-A*0201
			(SEQ. ID NO.:179)	
HBV	pol	642-650	ALMPLYACI	HLA-A*0201
			(SEQ. ID NO.:180)	
HBV	env	378-387	LLPIFFCLWV	HLA-A*0201
			(SEQ. ID NO.:181)	
HBV	pol	538-546	YMDDVVLGA	HLA-A*0201
			(SEQ. ID NO.:182)	
HBV	env	250-258	LLLCLIFLL	HLA-A*0201
			(SEQ. ID NO.:183)	
HBV	env	260-269	LLDYQGMLPV	HLA-A*0201
			(SEQ. ID NO.:184)	
HBV	env	370-379	SIVSPFIPLL	HLA-A*0201
			(SEQ. ID NO.:185)	
HBV	env	183-191	FLLTRILTI	HLA-A*0201
			(SEQ. ID NO.:186)	
HBV	cAg	88-96	YVNVNMGLK	HLA-A* 1101
			(SEQ. ID NO.:187)	
HBV	cAg	141-151	STLPETTVVRR	HLA-A*3101
			(SEQ. ID NO.:188)	
HBV	cAg	141-151	STLPETTVVRR	HLA-A*6801
			(SEQ. ID NO.:189)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*6801
			(SEQ. ID NO.:190)	
HBV	sAg	28-39	IPQSLDSWWTSL	H2-Ld
			(SEQ. ID NO.:191)	
HBV	cAg	93-100	MGLKFRQL	H2-Kb
			(SEQ. ID NO.:192)	
HBV	preS	141-149	STBXQSGXQ	HLA-A*0201

			(SEQ. ID NO.:193)	
HCMV	gp B	618-628	FIAGNSAYEYV	HLA-A*0201
			(SEQ. ID NO.:194)	
HCMV	E1	978-989	SDEEFAIVAYTL	HLA-B18
			(SEQ. ID NO.:195)	
HCMV	pp65	397-411	DDVWTSGSDSDEELV	HLA-b35
			(SEQ. ID NO.:196)	
HCMV	pp65	123-131	IPSINVHHY	HLA-B*3501
			(SEQ. ID NO.:197)	
HCMV	pp65	495-504	NLVPMVATVO	HLA-A*0201
			(SEQ. ID NO.:198)	
HCMV	pp65	415-429	RKTPRVTOGGAMAGA	HLA-B7
			(SEQ. ID NO.:199)	
HCV	MP	17-25	DLMGYIPLV	HLA-A*0201
			(SEQ. ID NO.:200)	
HCV	MP	63-72	LLALLSCLTV	HLA-A*0201
			(SEQ. ID NO.:201)	
HCV	MP	105-112	ILHTPGCV	HLA-A*0201
			(SEQ. ID NO.:202)	
HCV	env E	66-75	QLRRHIDLLV	HLA-A*0201
			(SEQ. ID NO.:203)	
HCV	env E	88-96	DLCGSVFLV	HLA-A*0201
			(SEQ. ID NO.:204)	
HCV	env E	172-180	SMVGNWAKV	HLA-A*0201
			(SEQ. ID NO.:205)	
HCV	NSI	308-316	HLIIQNIVDV	HLA-A*0201
			(SEQ. ID NO.:206)	
HCV	NSI	340-348	FLLLADARV	HLA-A*0201
			(SEQ. ID NO.:207)	
HCV	NS2	234-246	GLRDLAVAVEPVV	HLA-A*0201
			(SEQ. ID NO.:208)	
HCV	NSI	18-28	SLLAPGAKQNV	HLA-A*0201
			(SEQ. ID NO.:209)	
HCV	NSI	19-28	LLAPGAKQNV	HLA-A*0201
			(SEQ. ID NO.:210)	
HCV	NS4	192-201	LLFNILGGWV	HLA-A*0201
			(SEQ. ID NO.:211)	
HCV	NS3	579-587	YLVAYQATV	HLA-A*0201
			(SEQ. ID NO.:212)	
HCV	core protein	34-43	YLLPRRGPRL	HLA-A*0201
			(SEQ. ID NO.:213)	
HCV	MP	63-72	LLALLSCLTI	HLA-A*0201
			(SEQ. ID NO.:214)	
HCV	NS4	174-182	SLMAFTAAV	HLA-A*0201
			(SEQ. ID NO.:215)	
HCV	NS3	67-75	CINGVCWTV	HLA-A*0201
			(SEQ. ID NO.:216)	
HCV	NS3	163-171	LLCPAGHAV	HLA-A*0201
			(SEQ. ID NO.:217)	
HCV	NS5	239-247	ILDSFDPLV	HLA-A*0201
			(SEQ. ID NO.:218)	

HCV	NS4A	236-244	ILAGYGAGV	HLA-A*0201
			(SEQ. ID NO.:219)	
HCV	NS5	714-722	GLQDCTMLV	HLA-A*0201
			(SEQ. ID NO.:220)	
HCV	NS3	281-290	TGAPVTYSTY	HLA-A*0201
			(SEQ. ID NO.:221)	
HCV	NS4A	149-157	HMWNFISGI	HLA-A*0201
			(SEQ. ID NO.:222)	
HCV	NS5	575-583	RVCEKMALY	HLA-A*0201-A3
			(SEQ. ID NO.:223)	
HCV	NS1	238-246	TINYTIFK	HLA-A*1101
			(SEQ. ID NO.:224)	
HCV	NS2	109-116	YISWCLWW	HLA-A23
			(SEQ. ID NO.:225)	
HCV	core protein	40-48	GPRLGVRAT	HLA-B7
			(SEQ. ID NO.:226)	
HIV-1	gp120	380-388	SFNCGGEFF	HLA-Cw*0401
			(SEQ. ID NO.:227)	
HIV-1	RT	206-214	TEMEKEGKI	H2-Kk
			(SEQ. ID NO.:228)	
HIV-1	p17	18-26	KIRLRPGGK	HLA-A*0301
			(SEQ. ID NO.:229)	
HIV-1	P17	20-29	RLRPGGKKKY	HLA-A*0301
			(SEQ. ID NO.:230)	
HIV- I	RT	325-333	AIFQSSMTK	HLA-A*0301
			(SEQ. ID NO.:231)	
HIV-1	p17	84-92	TLYCVHQRI	HLA-A11
			(SEQ. ID NO.:232)	
HIV-1	RT	508-517	IYQEPFKNLK	HLA-Al1
			(SEQ. ID NO.:233)	
HIV-1	p17	28-36	KYKLKHIVW	HLA-A24
			(SEQ. ID NO.:234)	
HIV-1	gp120	53-62	LFCASDAKAY	HLA-A24
			(SEQ. ID NO.:235)	
HIV-1	gagp24	145-155	QAISPRTLNAW	HLA-A25
			(SEQ. ID NO.:236)	
HIV-1	gagp24	167-175	EVIPMFSAL	HLA-A26
			(SEQ. ID NO.:237)	
HIV-1	RT	593-603	ETFYVDGAANR	HLA-A26
			(SEQ. ID NO.:238)	
HIV-1	gp4l	775-785	RLRDLLLIVTR	HLA-A31
			(SEQ. ID NO.:239)	
HIV-1	RT	559-568	PIQKETWETW	HLA-A32
			(SEQ. ID NO.:240)	
HIV-1	gpl20	419-427	RIKQIINMW	HLA-A32
			(SEQ. ID NO.:241)	
HIV-1	RT	71-79	ITLWQRPLV	HLA-A*6802
			(SEQ. ID NO.:242)	
HIV-1	RT	85-93	DTVLEEMNL	HLA-A*6802
			(SEQ. ID NO.:243)	
HIV-1	RT	71-79	ITLWQRPLV	HLA-A*7401

			(SEQ. ID NO.:244)	
HIV-1	gag p24	148-156	SPRTLNAWV	HLA-B7
			(SEQ. ID NO.:245)	
HIV-1	gagp24	179-187	ATPQDLNTM	HLA-B7
			(SEQ. ID NO.:246)	
HIV-1	gpl20	303-312	RPNNNTRKSI	HLA-B7
			(SEQ. ID NO.:247)	
HIV-1	gp4l	843-851	IPRRIRQGL	HLA-B7
			(SEQ. ID NO.:248)	
HIV-1	p17	74-82	ELRSLYNTV	HLA-B8
			(SEQ. ID NO.:249)	
HIV-1	nef	13-20	WPTVRERM	HLA-B8
			(SEQ. ID NO.:250)	
HIV-1	nef	90-97	FLKEKGGL	HLA-B8
			(SEQ. ID NO.:251)	
HIV-1	gag p24	183-191	DLNTMLNTV	HLA-B14
			(SEQ. ID NO.:252)	
HIV-1	P17	18-27	KIRLRPGGKK	HLA-B27
			(SEQ. ID NO.:253)	
HIV-1	p17	19-27	IRLRPGGKK	HLA-B27
			(SEQ. ID NO.:254)	
HIV-1	gp4l	791-799	GRRGWEALKY	HLA-B27
			(SEQ. ID NO.:255)	
HIV-1	nef	73-82	QVPLRPMTYK	HLA-B27
			(SEQ. ID NO.:256)	
HIV-1	GP41	590-597	RYLKDQQL	HLA-B27
			(SEQ. ID NO.:257)	
HIV-1	nef	105-114	RRQDILDLWI	HLA-B*2705
			(SEQ. ID NO.:258)	
HIV-1	nef	134-141	RYPLTFGW	HLA-B*2705
			(SEQ. ID NO.:259)	
HIV-1	p17	36-44	WASRELERF	HLA-B35
			(SEQ. ID NO.:260)	
HIV-1	GAG P24	262-270	TVLDVGDAY	HLA-B35
			(SEQ. ID NO.:261)	
HIV-1	gp120	42-52	VPVWKEATTTL	HLA-B35
			(SEQ. ID NO.:262)	
HIV-1	P17	36-44	NSSKVSQNY	HLA-B35
			(SEQ. ID NO.:263)	
HIV-1	gag p24	254-262	PPIPVGDIY	HLA-B35
			(SEQ. ID NO.:264)	
HIV-1	RT	342-350	HPDIVIYQY	HLA-B35
			(SEQ. ID NO.:265)	
HIV-1	gp41	611-619	TAVPWNASW	HLA-B35
			(SEQ. ID NO.:266)	
HIV-1	gag	245-253	NPVPVGNIY	HLA-B35
			(SEQ. ID NO.:267)	
HIV-1	nef	120-128	YFPDWQNYT	HLA-B37
			(SEQ. ID NO.:268)	
HIV-1	gag p24	193-201	GHQAAMQML	HLA-B42
			(SEQ. ID NO.:269)	

HIV-1	p17	20-29	RLRPGGKKKY	HLA-B42
			(SEQ. ID NO.:270)	
HIV-1	RT	438-446	YPGIKVRQL	HLA-B42
			(SEQ. ID NO.:271)	
HIV-1	RT	591-600	GAETFYVDGA	HLA-B45
			(SEQ. ID NO.:272)	
HIV-1	gag p24	325-333	NANPDCKTI	HLA-B51
			(SEQ. ID NO.:273)	
HIV-1	gag p24	275-282	RMYSPTSI	HLA-B52
			(SEQ. ID NO.:274)	
HIV-1	gp120	42-51	VPVWKEATTT	HLA-B*5501
			(SEQ. ID NO.:275)	
HIV-1	gag p24	147-155	ISPRTLNAW	HLA-B57
			(SEQ. ID NO.:276)	
HIV-1	gag p24	240-249	TSTLQEQIGW	HLA-B57
			(SEQ. ID NO.:277)	
HIV-1	gag p24	162-172	KAFSPEVIPMF	HLA-B57
			(SEQ. ID NO.:278)	
HIV-1	gag p24	311-319	QASQEVKNW	HLA-B57
			(SEQ. ID NO.:279)	
HIV-1	gag p24	311-319	QASQDVKNW	HLA-B57
			(SEQ. ID NO.:280)	
HIV-1	nef	116-125	HTQGYFPDWQ	HLA-B57
			(SEQ. ID NO.:281)	
HIV-1	nef	120-128	YFPDWQNYT	HLA-B57
			(SEQ. ID NO.:282)	
HIV-1	gag p24	240-249	TSTLQEQIGW	HLA-B58
			(SEQ. ID NO.:283)	
HIV-1	p17	20-29	RLRPGGKKKY	HLA-B62
			(SEQ. ID NO.:284)	
HIV-1	p24	268-277	LGLNKJVRMY	HLA-B62
			(SEQ. ID NO.:285)	
HIV-1	RT	415-426	LVGKLNWASQIY	HLA-B62
			(SEQ. ID NO.:286)	
HIV-1	RT	476-485	ILKEPVHGVY	HLA-B62
			(SEQ. ID NO.:287)	
HIV-1	nef	117-127	TQGYFPDWQNY	HLA-B62
			(SEQ. ID NO.:288)	
HIV-1	nef	84-91	AVDLSHFL	HLA-B62
			(SEQ. ID NO.:289)	
HIV-1	gag p24	168-175	VIPMFSAL	HLA-Cw*0102
			(SEQ. ID NO.:290)	
HIV-1	gp120	376-384	FNCGGEFFY	HLA-A29
			(SEQ. ID NO.:291)	
HIV-1	gp120	375-383	SFNCGGEFF	HLA-B15
			(SEQ. ID NO.:292)	
HIV-1	nef	136-145	PLTFGWCYKL	HLA-A*0201
			(SEQ. ID NO.:293)	
HIV-1	nef	180-189	VLEWRFDSRL	HLA-A*0201
			(SEQ. ID NO.:294)	
HIV-1	nef	68-77	FPVTPQVPLR	HLA-B7

			(SEQ. ID NO.:295)	
HIV-1	nef	128-137	TPGPGVRYPL	HLA-B7
			(SEQ. ID NO.:296)	
HIV-1	gag p24	308-316	QASQEVKNW	HLA-Cw*0401
			(SEQ. ID NO.:297)	
HIV-1 IIIB	RT	273-282	VPLDEDFRKY	HLA-B35
			(SEQ. ID NO.:298)	
HIV-1 IIIB	RT	25-33	NPDIVIYQY	HLA-B35
			(SEQ. ID NO.:299)	
HIV-1 IIIB	gp41	557-565	RAIEAQAHL	HLA-B51
			(SEQ. ID NO.:300)	
HIV-1 IIIB	RT	231-238	TAFTIPSI	HLA-B51
			(SEQ. ID NO.:301)	
HIV- I IIIB	p24	215-223	VHPVHAGPIA	HLA-B*5501
			(SEQ. ID NO.:302)	
HIV-1 IIIB	gp120	156-165	NCSFNISTSI	HLA-Cw8
			(SEQ. ID NO.:303)	
HIV- I IIIB	gp120	241-249	CTNVSTVQC	HLA-Cw8
			(SEQ. ID NO.:304)	
HIV-1 5F2	gp120	312-320	IGPGRAFHT	H2-Dd
			(SEQ. ID NO.:305)	
HIV-1 5F2	pol	25-33	NPDIVIYQY	HLA-B*3501
			(SEQ. ID NO.:306)	
HIV-15F2	pol	432-441	EPIVGAETFY	HLA-B*3501
			(SEQ. ID NO.:307)	
HIV-1 5F2	pol	432-440	EPIVGAETF	HLA-B*3501
			(SEQ. ID NO.:308)	
HIV-1 5F2	pol	6-14	SPAIFQSSM	HLA-B*3501
			(SEQ. ID NO.:309)	
HIV-1 5F2	pol	59-68	VPLDKDFRKY	HLA-B*3501
			(SEQ. ID NO.:310)	
HIV-1 5F2	pol	6-14	IPLTEEAEL	HLA-B*3501
			(SEQ. ID NO.:311)	
HIV-1 5F2	nef	69-79	RPQVPLRPMTY	HLA-B*3501
			(SEQ. ID NO.:312)	
HIV-1 5F2	nef	66-74	FPVRPQVPL	HLA-B*3501
			(SEQ. ID NO.:313)	
HIV-1 5F2	env	10-18	DPNPQEVVL	HLA-B*3501
			(SEQ. ID NO.:314)	
HIV-1 5F2	env	7-15	RPIVSTQLL	HLA-B*3501
			(SEQ. ID NO.:315)	
HIV-1 5F2	pol	6-14	IPLTEEAEL	HLA-B51
			(SEQ. ID NO.:316)	
HIV-1 5F2	env	10-18	DPNPQEVVL	HLA-B51
			(SEQ. ID NO.:317)	
HIV-1 5F2	gagp24	199-207	AMQMLKETI	H2-Kd
			(SEQ. ID NO.:318)	
HIV-2	gagp24	182-190	TPYDrNQML	HLA-B*5301
			(SEQ. ID NO.:319)	
HIV-2	gag	260-269	RRWIQLGLQKV	HLA-B*2703
			(SEQ. ID NO.:320)	

HIV-1 5F2	gp41	593-607	GIWGCSGKLICTTAV	HLA-B17
			(SEQ. ID NO.:321)	
HIV-1 5F2	gp41	753-767	ALIWEDLRSLCLFSY	HLA-B22
			(SEQ. ID NO.:322)	
HPV 6b	E7	21-30	GLHCYEQLV	HLA-A*0201
			(SEQ. ID NO.:323)	
HPV 6b	E7	47-55	PLKQHFQIV	HLA-A*0201
			(SEQ. ID NO.:324)	
HPV11	E7	4-12	RLVTLKDIV	HLA-A*0201
			(SEQ. ID NO.:325)	
HPV16	E7	86-94	TLGIVCPIC	HLA-A*0201
			(SEQ. ID NO.:326)	
HPV16	E7	85-93	GTLGIVCPI	HLA-A*0201
			(SEQ. ID NO.:327)	
HPV16	E7	12-20	MLDLQPETT	HLA-A*0201
			(SEQ. ID NO.:328)	
HPV16	E7	11-20	YMLDLQPETT	HLA-A*0201
			(SEQ. ID NO.:329)	
HPVl6	E6	15-22	RPRKLPQL	HLA-B7
			(SEQ. ID NO.:330)	
HPV16	E6	49-57	RAHYNIVTF	HW-Db
			(SEQ. ID NO.:331)	
HSV	gp B	498-505	SSIEFARL	H2-Kb
			(SEQ. ID NO.:332)	
HSV-1	gp C	480-488	GIGIGVLAA	HLA-A*0201
			(SEQ. ID NO.:333)	
HSV-1	ICP27	448-456	DYATLGVGV	H2-Kd
			(SEQ. ID NO.:334)	
Virus	Protein		T cell epitope MHC ligand (Antigen)	MHC molecule
HSV-1	ICP27	322-332	LYRTFAGNPRA	H2-Kd
			(SEQ. ID NO.:335)	
HSV-1	UL39	822-829	QTFDFGRL	H2-Kb
			(SEQ. ID NO.:336)	
HSV-2	gpC	446-454	GAGIGVAVL	HLA-A*0201
			(SEQ. ID NO.:337)	
HLTV-1	TAX	11-19	LLFGYPVYV	HLA-A*0201
			(SEQ. ID NO.:338)	
Influenza	MP	58-66	GILGFVFTL	HLA-A*0201
			(SEQ. ID NO.:339)	
Influenza	MP	59-68	ILGFVFTLTV	HLA-A*0201
			(SEQ. ID NO.:340)	
Influenza	NP	265-273	ILRGSVAHK	HLA-A3
			(SEQ. ID NO.:341)	
Influenza	NP	91-99	KTGGPIYKR	HLA-A*6801
			(SEQ. ID NO.:342)	
Influenza	NP	380-388	ELRSRYWAI	HLA-B8
			(SEQ. ID NO.:343)	
Influenza	NP	381-388	LRSRYWAI	HLA-B*2702
			(SEQ. ID NO.:344)	
Influenza	NP	339-347	EDLRVLSFI	HLA-B*3701

			(SEQ. ID NO.:345)	
Influenza	NSI	158-166	GEISPLPSL	HLA-B44
			(SEQ. ID NO.:346)	
Influenza	NP	338-346	FEDLRVLSF	HLA-B44
			(SEQ. ID NO.:347)	
Influenza	NSI	158-166	GEISPLPSL	HLA-B*4402
			(SEQ. ID NO.:348)	
Influenza	NP	338-346	FEDLRVLSF	HLA-B*4402
			(SEQ. ID NO.:349)	
Influenza	PBI	591-599	VSDGGPKLY	HLA-Al
			(SEQ. ID NO.:350)	
Influenza A	NP	44-52	CTELKLSDY	HLA-Al
			(SEQ. ID NO.:351)	
Influenza	NSI	122-130	AIMDKNIIL	HLA-A*0201
			(SEQ. ID NO.:352)	
Influenza A	NSI	123-132	IMDKNIILKA	HLA-A*0201
			(SEQ. ID NO.:353)	
Influenza A	NP	383-391	SRYWAIRTR	HLA-B*2705
			(SEQ. ID NO.:354)	
Influenza A	NP	147-155	TYQRTRALV	H2-Kd
			(SEQ. ID NO.:355)	
Influenza A	HA	210-219	TYVSVSTSTL	H2-Kd
			(SEQ. ID NO.:356)	
Influenza A	HA	518-526	IYSTVASSL	H2-Kd
			(SEQ. ID NO.:357)	
Influenza A	HA	259-266	FEANGNLI	H2-Kk
			(SEQ. ID NO.:358)	
Influenza A	HA	10-18	IEGGWTGMI	H2-Kk
			(SEQ. ID NO.:359)	
Influenza A	NP	50-57	SDYEGRLI	H2-Kk
			(SEQ. ID NO.:360)	
Influenza a	NSI	152-160	EEGAIVGEI	H2-Kk
			(SEQ. ID NO.:361)	
Influenza A34	NP	336-374	ASNENMETM	H2Db
			(SEQ. ID NO.:362)	
Influenza A68	NP	366-374	ASNENMDAM	H2Db
			(SEQ. ID NO.:363)	
Influenza B	NP	85-94	KLGEFYNQMM	HLA-A*0201
			(SEQ. ID NO.:364)	
Influenza B	NP	85-94	KAGEFYNQMM	HLA-A*0201
			(SEQ. ID NO.:365)	
Influenza JAP	HA	204-212	LYQNVGTYV	H2Kd
			(SEQ. ID NO.:366)	
Influenza JAP	HA	210-219	TYVSVGTSTL	H2-Kd
			(SEQ. ID NO.:367)	
Influenza JAP	HA	523-531	VYQILATYA	H2-Kd
			(SEQ. ID NO.:368)	
Influenza JAP	HA	529-537	IYATVAGSL	H2-Kd
			(SEQ. ID NO.:369)	
Influenza JAP	HA	210-219	TYVSVGTSTI(L>I)	H2-Kd
			(SEQ. ID NO.:370)	

Influenza JAP	HA	255-262	FESTGNLI	H2-Kk
			(SEQ. ID NO.:371)	
JHMV	cAg	318-326	APTAGAFFF	H2-Ld
			(SEQ. ID NO.:372)	
LCMV	NP	118-126	RPQASGVYM	H2-Ld
			(SEQ. ID NO.:373)	
LCMV	NP	396-404	FQPQNGQFI	H2-Db
			(SEQ. ID NO.:374)	
LCMV	GP	276-286	SGVENPGGYCL	H2-Db
			(SEQ. ID NO.:375)	
LCMV	GP	33-42	KAVYNFATCG	H2-Db
			(SEQ. ID NO.:376)	
MCMV	pp89	168-176	YPHFMPTNL	H2-Ld
			(SEQ. ID NO.:377)	
MHV	spike protein	510-518	CLSWNGPHL	H2-Db
			(SEQ. ID NO.:378)	
MMTV	env gp 36	474-482	SFAVATTAL	H2-Kd
			(SEQ. ID NO.:379)	
MMTV	gag p27	425-433	SYETFISRL	H2-Kd
			(SEQ. ID NO.:380)	
MMTV	env gp73	544-551	ANYDFICV	H2-Kb
			(SEQ. ID NO.:381)	
MuLV	env p15E	574-581	KSPWFTTL	H2-Kb
			(SEQ. ID NO.:382)	
MuLV	env gp70	189-196	SSWDFITV	H2-Kb
			(SEQ. ID NO.:383)	
MuLV	gag 75K	75-83	CCLCLTVFL	H2-Db
			(SEQ. ID NO.:384)	
MuLV	env gp70	423-431	SPSYVYHQF	H2Ld
			(SEQ. ID NO.:385)	
MV	F protein	437-447	SRRYPDAVYLH	HLA-B*2705
			(SEQ. ID NO.:386)	
Mv	F protein	438-446	RRYPDAVYL	HLA-B*2705
Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule
			(SEQ. ID NO.:387)	
Mv	NP	281-289	YPALGLHEF	H2-Ld
			(SEQ. ID NO.:388)	
Mv	HA	343-351	DPVIDRLYL	H2-Ld
			(SEQ. ID NO.:389)	
MV	HA	544-552	SPGRSFSYF	H2-Ld
			(SEQ. ID NO.:390)	
Poliovirus	VP1	111-118	TYKDTVQL	H2-kd
			(SEQ. ID NO.:391)	
Poliovirus	VP1	208-217	FYDGFSKVPL	H2-Kd
			(SEQ. ID NO.:392)	
Pseudorabies virus gp	G111	455-463	IAGIGILAI	HLA-A*0201
			(SEQ. ID NO.:393)	
Rabiesvirus	NS	197-205	VEAEIAHQI	H2-Kk

			(SEQ. ID NO.:394)	
Rotavirus	VP7	33-40	llYRFLLl	H2-Kb
			(SEQ. ID NO.:395)	
Rotavirus	VP6	376-384	VGPVFPPGM	H2-Kb
			(SEQ. ID NO.:396)	
Rotavirus	VP3	585-593	YSGYIFRDL	H2-Kb
			(SEQ. ID NO.:397)	
RSV	M2	82-90	SYIGSINNI	H2-Kd
			(SEQ. ID NO.:398)	
SIV	gagp11C	179-190	EGCTPYDTNQML	Mamu-A*01
			(SEQ. ID NO.:399)	
SV	NP	324-332	FAPGNYPAL	H2-Db
			(SEQ. ID NO.:400)	
SV	NP	324-332	FAPCTNYPAL	H2-Kb
			(SEQ. ID NO.:401)	
SV40	T	404-411	VVYDFLKC	H2-Kb
			(SEQ. ID NO.:402)	
SV40	T	206-215	SAINNYAQKL	H2-Db
			(SEQ. ID NO.:403)	
SV40	T	223-231	CKGVNKEYL	H2-Db
			(SEQ. ID NO.:404)	
SV40	T	489-497	QGINNLDNL	H2-Db
			(SEQ. ID NO.:405)	
SV40	T	492-500 (501)	NNLDNLRDY(L)	H2-Db
			(SEQ. ID NO.:406)	
SV40	T	560-568	SEFLLEKRI	H2-Kk
			(SEQ. ID NO.:407)	
VSV	NP	52-59	RGYVYQGL	H2-Kb
			(SEQ. ID NO.:408)	

Table 2

HLA-A1	Position (Antigen)	Source
T cell epitopes	EADPTGHSY	MAGE-1 161-169
	(SEQ. ID NO.:409)	
	VSDGGPNLY	Influenza A PB 1591-599
	(SEQ. ID NO.:410)	
	CTELKLSDY	Influenza A NP 44-52
	(SEQ. ID NO.:411)	
	EVDPIGHLY	MAGE-3 168-176
	(SEQ. ID NO.:412)	
HLA-A201	MLLSVPLLLG	Calreticulin signal sequence I-10
	(SEQ, ID NO.:413)	
	STBXQSGXQ	HBV PRE-S PROTEIN 141-149
	(SEQ. ID NO.:414)	
	YMDGTMSQV	Tyrosinase 369-377
	(SEQ. ID NO.:415)	
	ILKEPVHGV	HIV- I RT 476-484
	(SEQ. ID NO.:416)	
	LLGFVFTLTV	Influenza MP 59-68
	(SEQ. ID NO.:417)	
	LLFGYPVYVV	HTLV-1 tax 11-19
	(SEQ. ID NO.:418)	
	GLSPTVWLSV	HBV sAg 348-357
	(SEQ. ID NO.:419)	
	WLSLLVPFV	HBV sAg 335-343
	(SEQ. ID NO.:420)	
	FLPSDFFPSV	HBV cAg 18-27
	(SEQ. ID NO.:421)	
	C L G 0 L L T M V	EBV LMP-2 426-434
	(SEQ. ID NO.:422)	
	FLAGNSAYEYV	HCMV gp 618-628B
	(SEQ. ID NO.:423)	
	KLGEFYNQMM	Influenza BNP 85-94
	(SEQ. ID NO.:424)	
	KLVALGINAV	HCV-1 NS3 400-409
	(SEQ. ID NO.:425)	
	DLMGYIPLV	HCV MP 17-25
	(SEQ. ID NO.:426)	
	RLVTLKDIV	HPV 11 EZ 4-12
	(SEQ. ID NO.:427)	
	MLLAVLYCL	Tyrosinase 1-9
	(SEQ. ID NO.:428)	
	AAGIGILTV	Melan A\Mart-127-35
	(SEQ. ID NO.:429)	
	YLEPGPVTA	Pmel 17/gp 100 480-488
	(SEQ. ID NO.:430)	
	ILDGTATLRL	Pmel 17/ gp 100 457-466

	(SEQ. ID NO.:431)	
	LLDGTATLRL	Pmel gplOO 457-466
	(SEQ. ID NO.:432)	
	ITDQVPFSV	Pmel gp 100 209-217
	(SEQ. ID NO.:433)	
	KTWGQYWQV	Pmel gp 100 154-162
	(SEQ. ID NO.:434)	
	TITDQVPFSV	Pmel gp 100 208-217
	(SEQ. ID NO.:435)	
	AFHIIVAREL	HIV- I nef 190-198
	(SEQ. ID NO.:436)	
	YLNKIQNSL	P. falciparum CSP 334-342
	(SEQ. ID NO.:437)	
	MMRKLAILSV	P. falciparum CSP 1 -10
	(SEQ. ID NO.:438)	
	KAGEFYNQMM	Influenza BNP 85-94
	(SEQ. ID NO.:439)	
	NIAEGLRAL	EBNA-1 480-488
	(SEQ. ID NO.:440)	
	NLRRGTALA	EBNA-1 519-527
	(SEQ. ID NO.:441)	
	ALAIPQCRL	EBNA-1 525-533
	(SEQ. ID NO.:442)	
	VLKDAIKDL	EBNA-1 575-582
	(SEQ. ID NO.:443)	
	FMVFLQTHI	EBNA-1 562-570
	(SEQ. ID NO.:444)	
	HLIVDTDSL	EBNA-2 15-23
	(SEQ. ID NO.:445)	
	SLGNPSLSV	EBNA-2 22-30
	(SEQ. ID NO.:446)	
	PLASAMRML	EBNA-2 126-134
	(SEQ. ID NO.:447)	
	RMLWMANYI	EBNA-2 132-140
	(SEQ. ID NO.:448)	
	MLWMANYIV	EBNA-2 133-141
	(SEQ. ID NO.:449)	
	ILPQGPQTA	EBNA-2 151-159
	(SEQ. ID NO.:450)	
	PLRPTAPTTI	EBNA-2 171-179
	(SEQ. ID NO.:451)	
	PLPPATLTV	EBNA-2 205-213
	(SEQ. ID NO.:452)	
	R M H L P V L H V	EBNA-2 246-254
	(SEQ. ID NO.:453)	
	PMPLPPSQL	EBNA-2 287-295
	(SEQ. ID NO.:454)	
	QLPPPAAPA	EBNA-2 294-302

	(SEQ. ID NO.:455)	
	SMPELSPVL	EBNA-2 381-389
	(SEQ. ID NO.:456)	
	DLDESWDYI	EBNA-2 453-461
	(SEQ. ID NO.:457)	
	P L P C V L W P VV	BZLFl 43-51
	(SEQ. ID NO.:458)	
	SLEECDSEL	BZLFl 167-175
	(SEQ, ID NO.:459)	
	EIKRYKNRV	BZLFI 176-184
	(SEQ. ID NO.:460)	
	QLLQFIYREV	BZLFl 195-203
	(SEQ. ID NO.:461)	
	LLQHYREVA	BZLFI 196-204
	(SEQ. ID NO.:462)	
	LLKQMCPSL	BZLFI 217-225
	(SEQ. ID NO.:463)	
	SIIPRTPDV	BZLFI 229-237
	(SEQ. ID NO.:464)	
	AIMDKNIIL	Influenza A NS1 122-130
	(SEQ. ID NO.:465)	
	IMDKNIILKA	Influenza A NS1 123-132
	(SEQ. ID NO.:466)	
	LLALLSCLTV	HCV MP 63-72
	(SEQ. ID NO.:467)	
	ILHTPGCV	HCV MP 105-112
	(SEQ. ID NO.:468)	
	QLRRHIDLLV	HCV env E 66-75
	(SEQ, ID NO.:469)	
	DLCGSVFLV	HCV env E 88-96
	(SEQ. ID NO.:470)	
	SMVGNWAKV	HCV env E 172-180
	(SEQ. ID NO.:471)	
	HLHQNIVDV	HCV NSI 308-316
	(SEQ. ID NO.:472)	
	FLLLADARV	HCV NSI 340-348
	(SEQ. ID NO.:473)	
	GLRDLAVAVEPVV	HCV NS2 234-246
	(SEQ. ID NO.:474)	
	SLLAPGAKQNV	HCV NS1 18-28
	(SEQ. ID NO.:475)	
	LLAPGAKQNV	HCV NS1 19-28
	(SEQ. ID NO.:476)	
	FLLSLGIHL	HBV pol 575-583
	(SEQ. ID NO.:477)	
	SLYADSPSV	HBV pol 816-824
	(SEQ. ID NO.:478)	
	GLSRYVARL	HBV POL 455-463

	(SEQ. ID NO.:479)	
	KIFGSLAFL	HER-2 369-377
	(SEQ. ID NO.:480)	
	ELVSEFSRM	HER-2 971-979
	(SEQ. ID NO.:481)	
	KLTPLCVTL	HIV- I gp 160 120-128
	(SEQ. ID NO.:482)	
	SLLNATDIAV	HIV- I GP 160 814-823
	(SEQ. ID NO.:483)	
	VLYRYGSFSV	Pmel gpl00 476-485
	(SEQ. ID NO.:484)	
	YIGEVLVSV	Non-filament forming class I myosin family (HA-2)**
	(SEQ. ID NO.:485)	
	LLFNILGGWV	HCV NS4 192-201
	(SEQ. ID NO.:486)	
	LLVPFVQWFW	HBV env 338-347
	(SEQ. ID NO.:487)	
	ALMPLYACI	HBV pol 642-650
	(SEQ. ID NO.:488)	
	YLVAYQATV	HCV NS3 579-587
	(SEQ. ID NO.:489)	
	TLGIVCPIC	HIPV 16 E7 86-94
	(SEQ. ID NO.:490)	
	YLLPRRGPRL	HCV core protein 34-43
	(SEQ. ID NO.:491)	
	LLPIFFCLWV	HBV env 378-387
	(SEQ. ID NO.:492)	
	YMDDVVLGA	HBV Pol 538-546
	(SEQ. ID NO.:493)	
	GTLGIVCPI	HPV16 E7 85-93
	(SEQ. ID NO.:494)	
	LLALLSCLTI	HCV MP 63-72
	(SEQ. ID NO.:495)	
	MLDLQPETT	HPV 16 E7 12-20
	(SEQ. ID NO.:496)	
	SLMAFTAAV	HCV NS4 174-182
	(SEQ. ID NO.:497)	
	CINGVCWTV	HCV NS3 67-75
	(SEQ. ID NO.:498)	
	VMNILLQYVV	Glutarnic acid decarboxylase 114-123
	(SEQ. ID NO.:499)	
	ILTVILGVL	Melan A/Mart- 32-40
	(SEQ. ID NO.:500)	
	FLWGPRALV	MAGE-3 271-279
	(SEQ. ID NO.:501)	
	L L C P A G H A V	HCV NS3 163-171
	(SEQ. ID NO.:502)	

	ILDSFDPLV	HCV NSS 239-247
	(SEQ. ID NO.:503)	
	LLLCLIFLL	HBV env 250-258
	(SEQ. ID NO.:504)	
	LIDYQGMLPV	HBV env 260-269
	(SEQ. ID NO.:505)	
	SIVSPFIPLL	HBV env 370-379
	(SEQ. ID NO.:506)	
	FLLTRILTI	HBV env 183-191
	(SEQ. ID NO.:507)	
	HLGNVKYLV	P. faciparum TRAP 3-11
	(SEQ. ID NO.:508)	
	GIAGGLALL	P. faciparum TRAP 500-508
	(SEQ. ID NO.:509)	
	ILAGYGAGV	HCV NS S4A 236-244
	(SEQ. ID NO.:510)	
	GLQDCTMLV	HCV NS5 714-722
	(SEQ. ID NO.:511)	
	TGAPVTYSTY	HCV NS3 281-290
	(SEQ. ID NO.:512)	
	VIYQYMDDLV	HIV-1RT 179-187
	(SEQ. ID NO.:513)	
	VLPDVFIRCV	N-acetylglucosaminyltransferase V Gnt-V intron
	(SEQ. ID NO.:514)	
	VLPDVFIRC	N-acetylglucosaminyltransferase V Gnt-V intron
	(SEQ. ID NO.:515)	
	AVGIGIAVV	Human CD9
	(SEQ. ID NO.:516)	
	LVVLGLLAV	Human glutamyltransferase
	(SEQ. ID NO.:517)	
	ALGLGLLPV	Human G protein coupled receptor
	(SEQ. ID NO.:518) 164-172	
	GIGIGVLAA	HSV- I gp C 480-488
	(SEQ. ID NO.:519)	
	GAGIGVAVL	HSV-2 gp C 446-454
	(SEQ. ID NO.:520)	
	IAGIGILAI	Pseudorabies gpGIN 455-463
	(SEQ. ID NO.:521)	
	LIVIGILIL	Adenovirus 3 E3 9kD 30-38
	(SEQ. ID NO.:522)	
	LAGIGLIAA	S. Lincolnensis ImrA
	(SEQ. ID NO.:523)	
	VDGIGILTI	Yeast ysa-1 77-85
	(SEQ. ID NO.:524)	
	GAGIGVLTA	B. polymyxa, βcndoxylanase 149- 157

	(SEQ. ID NO.:525) 157	
	AAGIGIIQI	E. coli methionine synthase 590-598
	(SEQ. ID NO.:526)	
	QAGIGILLA	E. coli hypothetical protein 4-12
	(SEQ. ID NO.:527)	
	KARDPHSGHFV	CDK4wl 22.32
	(SEQ. ID NO.:528)	
	KACDPI-ISGIIFV	CDK4-R24C 22-32
	(SEQ. ID NO.:529)	
	ACDPFISGHFV	CDK4-R24C 23-32
	(SEQ. ID NO.:530)	
	SLYNTVATL	HIV- I gag p 17 77-85
	(SEQ. ID NO.:531)	
	ELVSEFSRV	HER-2, m>V substituted 971-979
	(SEQ. ID NO.:532)	
	RGPGRAFVTI	HIV- I gp 160 315-329
	(SEQ. ID NO.:533)	
	HMWNFISGI	HCV NS4A 149-157
	(SEQ. ID NO.:534)	
	NLVPMVATVQ	HCMV pp65 495-504
	(SEQ. ID NO.:535)	
	GLHCYEQLV	HPV 6b E7 21-30
	(SEQ. ID NO.:536)	
	PLKQHFQIV	HPV 6b E7 47-55
	(SEQ. ID NO.:537)	
	LLDFVRFMGV	EBNA-6 284-293
	(SEQ. ID NO.:538)	
	AIMEKNIML	Influenza Alaska NS 1 122-130
	(SEQ. ID NO.:539)	
	YLKTIQNSL	P. falciparum cp36 CSP
	(SEQ. ID NO.:540)	
	YLNKIQNSL	P. falciparum cp39 CSP
	(SEQ. ID NO.:541)	
	YMLDLQPETT	HPV 16 E7 11-20*
	(SEQ. ID NO.:542)	
	LLMGTLGIV	HPV16 E7 82-90**
	(SEQ. ID NO.:543)	
	TLGIVCPI	HPV 16 E7 86-93
	(SEQ. ID NO.:544)	
	TLTSCNTSV	HIV-1 gp120 197-205
	(SEQ. ID NO.:545)	
	KLPQLCTEL	HPV 16 E6 18-26
	(SEQ. ID NO.:546)	
	TIHDIILEC	HPV16 E6 29-37
	(SEQ. ID NO.:547)	
	LGIVCPICS	HPV16 E7 87-95
	(SEQ. ID NO.:548)	

	VILGVLLLI	Melan A/Mart-1 35-43
	(SEQ. ID NO.:549)	
	ALMDKSLHV	Melan A/Mart- 1 56-64
	(SEQ. ID NO.:550)	
	GILTVILGV	Melan A/Mart- 1 31-39
	(SEQ. ID NO.:551)	
T cell epitopes	MINAYLDKL	P. Falciparum STARP 523-531
	(SEQ. ID NO.:552)	
	AAGIGILTV	Melan A/Mart- 127-35
	(SEQ. ID NO.:553)	
	FLPSDFFPSV	HBV cAg 18-27
	(SEQ. ID NO.:554)	
Motif unknown	SVRDRLARL	EBNA-3 464-472
T cell epitopes	(SEQ. ID NO.:555)	
T cell epitopes	AAGIGILTV	Melan A/Mart-1 27-35
	(SEQ. ID NO.:556)	
	FAYDGKDYI	Human MHC I-ot 140-148
	(SEQ. ID NO.:557)	
T cell epitopes	AAGIGILTV	Melan A/Mart-1 27-35
	(SEQ. ID NO.:558)	
	FLPSDFFPSV	HBV cAg 18-27
	(SEQ. ID NO.:559)	
Motif unknown	AAGIGILTV	Meland A/Mart-1 27-35
T cell epitopes	(SEQ. ID NO.:560)	
	FLPSDFFPSV	HBV cAg 18-27
	(SEQ. ID NO.:561)	
	AAGIGILTV	Melan A/Mart-1 27-35
	(SEQ. ID NO.:562)	
	ALLAVGATK	Pmel17 gp 100 17-25
	(SEQ. ID NO.:563)	
T cell epitopes	R L R D L L L I V T R	HIV-1 gp41 768-778
	(SEQ. ID NO.:564)	
	QVPLRPMTYK	HIV-1 nef 73-82
	(SEQ. ID NO.:565)	
	TVYYGVPVWK	HIV-1 gp120-36-45
	(SEQ. ID NO.:566)	
	RLRPGGKKK	HIV- 1 gag p 17 20-29
	(SEQ. ID NO.:567)	
	ILRGSVAHK	Influenza NP 265-273
	(SEQ. ID NO.:568)	
	RLRAEAGVK	EBNA-3 603-611
	(SEQ. ID NO.:569)	
	RLRDLLLIVTR	HIV-1 gp41 770-780
	(SEQ. ID NO.:570)	
	VYYGVPVWK	HIV- I GP 120 38-46
	(SEQ. ID NO.:571)	
	RVCEKMALY	HCV NS5 575-583
	(SEQ. ID NO.:572)	

Motif unknown	KIFSEVTLK	Unknown; muta melanoma peptide ted (p I 83L) 175-183
T cell epitope	(SEQ. ID NO.:573)	
	YVNVNMGLK*	HBV cAg 88-96
	(SEQ. ID NO.:574)	
T cell epitopes	IVTDFSVIK	EBNA-4 416-424
	(SEQ. ID NO.:575)	
	ELNEALELK	P53 343-351
	(SEQ. ID NO.:576)	
	VPLRPMTYK	HIV- 1 NEF 74-82
	(SEQ. ID NO.:577)	
	AIFQSSMTK	HIV- I gag p24 325-333
	(SEQ. ID NO.:578)	
	QVPLRPMTYK	HIV-1 nef 73-82
	(SEQ. ID NO.:579)	
	TINYTIFK HCV	NSI 238-246
	(SEQ. ID NO.:580)	
	AAVDLSHFLKEK	HIV-1 nef 83-94
	(SEQ. ID NO.:581)	
	ACQ G V G G P G G H K	HIV-1 I I 1B p24 349-359
	(SEQ. ID NO.:581)	
HLA-A24	S Y L D S G I H F*	β-catenin, mutated (proto-onocogen) 29-37
	(SEQ. ID NO.:582)	
T cell epitopes	RYLKDQQLL	HIV GP 41 583-591
	(SEQ. ID NO.:583)	
	AYGLDFYIL	P15 melanoma Ag 10- 18
	(SEQ. ID NO.:584)	
	AFLPWHRLFL	Tyrosinase 206-215
	(SEQ. ID NO.:585)	
	AFLPWHRLF	Tyrosinase 206-214
	(SEQ. ID NO.:586)	
	RYSIFFDY	Ebna-3 246-253
	(SEQ. ID NO.:587)	
T cell epitope	ETINEEAAEW	HIV- 1 gag p24 203-212
	(SEQ. ID NO.:588)	
T cell epitopes	STLPETTVVRR	HBV cAg 141 -151
	(SEQ. ID NO.:589)	
	MSLQRQFLR	ORF 3P-gp75 294-321 (bp)
	(SEQ. ID NO.:590)	
	LLPGGRPYR	TRP (tyrosinase rel.) 197-205
	(SEQ. ID NO.:591)	
T cell epitope	IVGLNKIVR	HIV gag p24 267-267-275
	(SEQ. ID NO.:592)	
	AAGIGILTV	Melan A/Mart- 127 35
	(SEQ. ID NO.:593)	

Table 3 sets forth additional antigens useful in the invention that are available from the Ludwig Cancer Institute. The Table refers to patents in which the identified antigens can be found. TRA refers to the tumor-related antigen and the LUD No. refers to the Ludwig Institute number.

Table 3

TRA	LUD No.	Patent No.	Date Patent Issued	Peptide (Antigen)	HLA
MAGE-4	5293	5,405,940	11 April 1995	EVDPASNTY	HLA-Al
				(SEQ. ID NO.:532)	
MAGE-41	5293	5,405,940	11 April 1995	EVDPTSNTY	HLA-A 1
				(SEQ ID NO:533)	
MAGE-5	5293	5,405,940	11 April 1995	EADPTSNTY	HLA-A 1
				(SEQ ID NO:534)	
MAGE-51	5293	5,405,940	11 April 1995	EADPTSNTY	HLA-A 1
				(SEQ ID NO:534)	
MAGE-6	5294	5,405,940	11 April 1995	EVDPIGHVY	HLA-Al
				(SEQ ID NO:535)	
	5299.2	5,487,974	30 January 1996	MLLAVLYCLL	HLA-A2
				(SEQ ID NO:536)	
	5360	5,530,096	25 June 1996	MLLAVLYCL	HLA-B44
				(SEQ ID NO:537)	
Tyrosinase	5360.1	5,519,117	21 May 1996	SEIWRDIDFA	HLA-B44
				(SEQ ID NO:538)	
				SEIWRDIDF	
				(SEQ ID NO:539)	
Tyrosinase	5431	5,774,316	28 April 1998	XEIWRDIDF	HLA-B44
				(SEQ ID NO:540)	
MAGE-2	5340	5,554,724	10 September 1996	STLVEVTLGEV	HLA-A2
				(SEQ ID NO:541)	
				LVEVTLGEV	
				(SEQ ID NO:542)	
				VIFSKASEYL	
				(SEQ ID NO:543)	
				IIVLAIIAI	
				(SEQ ID NO:544)	
(Continued)				KIWEELSMLEV	
				(SEQ ID NO:545)	
TRA	**LUD No.**	**Patent No.**	**Date Patent Issued**	**Peptide (Antigen)**	**HLA**

				LIETSYVKV	
				(SEQ ID NO:546)	
	5327	5,585,461	17 December 1996	FLWGPRALV	HLA-A2
				(SEQ ID NO: 547)	
				TLVEVTLGEV	
				(SEQ ID NO:548)	
				ALVETSYVKV	
				(SEQ ID NO:549)	
MAGE-3	5344	5,554,506	10 September 1996	KIWEELSVL	HLA-A2
				(SEQ ID NO:550)	
MAGE-3	5393	5,405,940	11 April 1995	EVDPIGHLY	HLA-Al
				(SEQ ID NO:551)	
MAGE	5293	5,405,940	11 April 1995	EXDX5Y	HLA-Al
				(SEQ. ID NO.:552)	
				(but not EADPTGHSY)	
				(SEQ. ID NO.:553)	
				E (A/V) D X5 Y	
				(SEQ. ID NO.:554)	
				E (A/V) D P X4 Y	
				(SEQ. ID NO.:555)	
				E (A/V) D P (I/A/T) X3 Y	
				(SEQ. ID NO.:556)	
				E (A/V) D P (I/A/T) (G/S) X2 Y	
				(SEQ. ID NO.:557)	
				E (A/V) D P (I/A/T) (G/S) (H/N) X Y	
				E (A/V) DP (I/A/T) (G/S) (H/N) (L/T/V) Y	
				(SEQ. 11) NO.:559)	
MAGE-1	5361	5,558.995	24 September 1996	ELHSAYGEPRKLLTQD	HLA-C
				(SEQ ID NO:560)	Clone 10
				EHSAYGEPRKLL	
				(SEQ ID NO:561)	
				SAYGEPRKL	
				(SEQ ID NO:562)	
MAGE-1	5253.4	TBA	TBA	EADPTGHSY	HLA-A 1
				(SEQ ID NO:563)	

TRA	LUD No.	Patent No.	Date Patent Issued	Peptide (Antigen)	HLA
BAGE	5310.1	TBA	TBA	MAARAVFLALSAQLLQARLMKE	HLA-C
				(SEQ ID NO:564)	Clone 10
				MAARAVFLALSAQLLQ	HLA-C
				(SEQ ID NO:565)	Clone 10
				AARAVFLAL	HLA-C
				(SEQ ID NO:566)	Clone 10
GAGE	5323.2	5,648,226	15 July 1997	YRPRPRRY	HLA-CW6
				(SEQ. ID NO.:567)	

Table 4

Source	Protein	AA Position	MHC molecules	T cell epitope MHC ligand (Antigen)	Ref.
synthetic peptides	synthetic peptides	synthetic peptides	HLA-A2	ALFAAAAAV	Parker, et al., "Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains," J. Immunol. 152:163-175
			"	GIFGGVGGV	"
			"	GLDKGGGV	"
			"	GLFGGFGGV	"
			"	GLFGGGAGV	"
			"	GLFGGGEGV	"
			"	GLFGGGFGV	"
			"	GLFGGGGGL	"
			"	GLFGGGGGV	"
			"	GLFGGGVGV	"
			"	GLFGGVGGV	"
			"	GLFGGVGKV	"
			"	GLFKGVGGV	"
			"	GLGGGGFGV	"
			"	GLLGGGVGV	"
			"	GLYGGGGGV	"
			"	GMFGGGGGV	"
			"	GMFGGVGGV	"
			"	GQFGGVGGV	"
			"	GVFGGVGGV	"
			"	KLFGGGGGV	"
			"	KLFGGVGGV	"
			"	AILGFVFTL	"
			"	GAIGFVFTL	"
			"	GALGFVFTL	"
			"	GELGFVFTL	"
			"	GIAGFVFTL	"
			"	GIEGFVFTL	"
			"	GILAFVFTL	"
			"	GILGAVFTL	"
			"	GILGEVFTL	"

			"	GILFGAFTL	"
			"	GILGFEFTL	"
			"	GILGFKFTL	"
			"	GILGFVATL	"
			"	GILGFVETL	"
			"	GILGFVFAL	"
			"	GILGFVFEL	"
			"	GILGFVFKL	"
			"	GILGFVFTA	"
			"	GILGFVFTL	"
			"	GILGFVFVL	"
			"	GILGFVKTL	"
			"	GILGKVFTL	"
			"	GILKFVFTL	"
			"	GILPFVFTL	"
			"	GIVGFVFTL	"
			"	GKLGFVFTL	"
			"	GLLGFVFTL	"
			"	GQLGFVFTL	"
			"	KALGFVFTL	"
			"	KILGFVFTL	"
			"	KILGKVFTL	"
			"	AILLGVFML	"
			"	AIYKRWIIL	"
			"	ALFFFDIDL	"
			"	ATVELLSEL	"
			"	CLFGYPVYV	"
			"	FIFPNYTIV	"
			"	IISLWDSQL	"
			"	ILASLFAAV	"
			"	ILESLFAAV	"
			"	KLGEFFNQM	"
			"	KLGEFYNQM	"
			"	LLFGYPVYV	"
			"	LLWKGEGAV	"
			"	LMFGYPVYV	"
			"	LNFGYPVYV	"
			"	LQFGYPVYV	"
			"	NIVAHTFKV	"
			"	NLPMVATV	"
			"	QMLLAIARL	"
			"	QMWQARLTV	"
			"	RLLQTGIHV	"
			"	RLVNGSLAL	"
			"	SLYNTVATL	"
			"	TLNAWVKVV	"
			"	WLYRETCNL	"

			"	YLFKRMIDL	"
			"	GAFGGVGGV	"
			"	GAFGGVGGY	"
			"	GEFGGVGGV	"
			"	GGFGGVGGV	"
			"	GIFGGGGGV	"
			"	GIGGFGGGL	"
			"	GIGGGGGGL	"
			"	GLDGGGGGV	"
			"	GLDGKGGGV	"
			"	GLDKKGGGV	"
			"	GLFGGGFGF	"
			"	GLFGGGFGG	"
			"	GLFGGGFGN	"
			"	GLFGGGFGS	"
			"	GLFGGGGGI	"
			"	GLFGGGGGM	"
			"	GLFGGGGGT	"
			"	GLFGGGGGY	"
			"	GLGFGGGGV	"
			"	GLGGFGGGV	"
			"	GLGGGFGGV	"
			"	GLGGGGGFV	"
			"	GLGGGGGGY	"
			"	GLGGGVGGV	"
			"	GLLGGGGGV	"
			"	GLPGGGGGV	"
			"	GNFGGVGGV	"
			"	GSFGGVGGV	"
			"	GTFGGVGGV	"
			"	AGNSAYEYV	"
			"	GLFPGQFAY	"
			"	HILLGVFML	"
			"	ILESLFRAV	"
			"	KKKYKLKHI	"
			"	MLASIDLKY	"
			"	MLERELVRK	"
			"	KLFGFVFTV	"
			"	ILDKKVEKV	"
			"	ILKEPVHGV	"
			"	ALFAAAAAY	"
			"	GIGFGGGGL	"
			"	GKFGGVGGV	"
			"	GLFGGGGGK	"
			"	EILGFVFTL	"
			"	GIKGFVFTL	"
			"	GQLGFVFTK	"

			"	ILGFVFTLT	"
			"	KILGFVFTK	"
			"	KKLGFVFTL	"
			"	KLFEKVYNY	"
			"	LRFGYPVYV	"
Human	HSP60	140-148	HLA-B27	IRRGVMLAV	Rammensee et al. 1997 160
"	"	369-377	"	KRIQEIIEQ	"
"	"	469-477	"	KRTLKIPAM	"
Yersinia	HSP60	35-43	"	GRNVVLDKS	"
"	"	117-125	"	KRGIDKAVI	"
"	"	420-428	"	IRAASAITA	"
"	HSP 60	284-292	HLA-B*2705	RRKAMFEDI	169
P.falciparum	LSA-1	1850-1857	HLA-B3501	KPKDELDY	170
Influenza NP		379-387	HLA-B*4402	LELRSRYWA	183
	Tum-P35B	4-13	HLA-D^d	GPPHSNNFGY	230
Rotavirus	VP7	33-40		IIYRFLLI	262
	OGDH (F108Y)	104-112	H2-L^d	QLSPYPFDL	253
	TRP-2	181-188	p287	VYDFFVWL	284
	DEAD box p 68	547-554	p287	SNFVFAGI	283
	Vector "artefact"		p287	SVVEFSSL	260

	Epiope mimic of tumor Ag		p287	AHYLFRNL	278
			"	THYLFRNL	"
	Epitope mimic of H-3 miHAg"		"	LIVIYNTL	279
			"	LIYEFNTL	"
			"	IPYIYNTL	"
			"	IIYIYHRL	"
			"	LIYIFNTL	"
	HBV cAg	93-100	"	MGLKFRQL	280
Human	autoantigen LA	51-58	"	IMIKFRNRL	281
Mouse	UTY protein		$H2D^b$	WMHHNMDLI	303
Mouse	p53	232-240	"	KYMCNSSCM	302
MURINE	MDM2	441-449	"	GRPKNGCIV	277
	Epitope mimic of natural		"	AQHPNAELL	278
	MuLV gag75K	75-83	"	CCLCLTVFL	301
P. Falciparum	CSP	375-383	p290	YENDIEKK	315
"	"	371-379	"	DELDYENDI	315
HIV	-1RT	206-214	"	TEMEKEGKI	316
Rabies	NS	197-205		VEAEIAHQI	309, 310
Influenza A	NS1	152-160	"	EEGAIVGEI	304
Murine	SMCY		p291	TENSGKDI	317
	MHC class 1 leader	3-11	p293	AMAPRTLLL	318
	ND1alpha	1-12	p293	FFINILTLLVP	323
	ND Beta	1-12	p293	FFINILTLLVP	323
	ND alpha	1-17	"	FFINILTLLVPILIAM	324
	ND Beta	1-17	"	FFINALTLLVPILIAM	"
	COI mitochondrial	1-6	"	FINRW	325

L. monocytogenes	LemA	1-6	"	IGWII	326
	SIV gag p11C	179-190	Mamu-A*01	EGCTPYDINQML	334
	MAGE-3		HLA-A2	ALSRKVAEL	5,554,506
			"	IMPKAGLLI	"
			"	KIWEELSVL	"
			"	ALVETSYVKV	"
			"	ThrLeuValGluValThrLeuGlyGluVal	"
			"	AlaLeuSerArgLysValAlaGluLeu	"
			"	IleMetProLysAlaGlyLeuLeuIle	"
			"	LysIleTrpGluGluLeuSerValLeu	"
			"	AlaLeuValGluThrSerTyrValLysVal	"
	peptides which bind to MHCs		HLA-A2	Lys Gly Ile Leu Gly Phe Val Phe Thr Leu Thr Val	5,989,565
			"	Gly Ile Ile Gly Phe Val Phe Thr Ile	"
			"	Gly Ile Ile Gly Phe Val Phe Thr Leu	"
			"	Gly Ile Leu Gly Phe Val Phe Thr Leu	"
			"	Gly Leu Leu Gly Phe Val Phe Thr Leu	"
			"	XXTVXXGVX, X=Leu or Ile (6-37)	"
			"	Ile Leu Thr Val Ile Leu Gly Val Leu	"
			"	Tyr Leu Glu Pro Gly Pro Val Thr Ala	"
			"	Gln Val Pro Leu Arg Pro Met Thr Tyr Lys	"

			"	Asp Gly Leu Ala Pro Pro Gln His Leu Ile Arg	"
			"	Leu Leu Gly Arg Asn Ser Phe Glu Val	"
	Peptides from MAGE-1		HLA-C clone 10	GluHisSerAlaTyrGlyGluProArgLysLeuLeuThrGlnAspLeu	5,558,995
			"	GluHisSerAlaTyrGlyGluProArgLysLeuLeu	"
			"	SerAlaTyrGlyGluProArgLysLeu	"
	GAGE		HLA-Cw6	TyrArgProArgProArgArgTyr	5,648,226
			"	ThrTyrArgProArgProArgArgTyr	"
			"	TyrArgProArgProArgArgTyrVal	"
			"	ThrTyrArgProArgProArgArgTyrVal	"
			"	ArgProArgProArgArgTyrValGlu	"
			"	MetSerTrpArgGlyArgSerThrTyrArgProArgProArgArg	"
			"	ThrTyrArgProArgProArgArgTyrValGluProProGluMetIle	"
	MAGE		HLA-A1, primarily	Isolated nonapeptide having Glu at its N terminal, Tyr at its C-terminal, and Asp at the third residue from its N terminal, with the proviso that said isolated nonapeptide is not Glu Ala Asp Pro Thr Gly His Ser Tyr (SEQ ID NO: 1), and wherein said isolated nonapeptide binds to a human leukocyte antigen molecule on a cell to form a complex, said complex provoking lysis of said	5,405,940

				cell by a cytolytic T cell specific to said complex	
			"	GluValValProIleSerHis LeuTyr	"
			"	GluValValArgIleGlyHi sLeuTyr	"
			"	GluValAspProIleGlyHi sLeuTyr	"
			"	GluValAspProAlaSerA snThrTyr	"
			"	GluValAspProThrSerA snThrTyr	"
			"	GluAlaAspProThrSerA snThrTyr	"
			"	GluValAspProIleGlyHi sValTyr	"
			"	GAAGTGGTCCCCAT CAGCCACTTGTAC	"
			"	GAAGTGGTCCGCA TCGGCCACTTGTAC	"
			"	GAAGTGGACCCCA TCGGCCACTTGTAC	"
			"	GAAGTGGACCCCG CCAGCAACACCTAC	"
			"	GAAGTGGACCCCA CCAGCAACACCTAC	"
			"	GAAGCGGACCCCA CCAGCAACACCTAC	"
			"	GAAGCGGACCCCA CCAGCAACACCTAC	"
			"	GAAGTGGACCCCA TCGGCCACGTGTAC	"
			"	GluAlaAspProThrGly HisSer	"
			"	AlaAspProTrpGlyHisS erTyr	"
	MAGE peptides		HLA-A2	SerThrLeuValGluValT hrLeuGlyGluVal	5,554,724
	"		"	LeuValGluValThrLeu GlyGluVal	"
	"		"	LysMetValGluLeuVal HisPheLeu	"
	"		"	ValIlePheSerLysAlaSe rGluTyrLeu	"
	"		"	TyrLeuGlnLeuValPhe GlyIleGluVal	"

	"		"	GlnLeuValPheGlyIleGluValVal	"
	"		"	GlnLeuValPheGlyIleGluValValGluVal	"
	"		"	IleIleValLeuAlaIleIleAlaIle	"
	"		"	LysIleTrpGluGluLeuSerMetLeuGluVal	"
	"		"	AlaLeuIleGluThrSerTyrValLysVal	"
	"		"	LeuIleGluThrSerTyrValLysVal	"
	"		"	GlyLeuGluAlaArgGlyGluAlaLeuGlyLeu	"
	"		"	GlyLeuGluAlaArgGlyGluAlaLeu	"
	"		"	AlaLeuGlyLeuValGlyAlaGlnAla	"
	"		"	GlyLeuValGlyAlaGlnAlaProAla	"
	"		"	AspLeuGluSerGluPheGlnAlaAla	"
	"		"	AspLeuGluSerGluPheGlnAlaAlaIle	"
	"		"	AlaIleSerArgLysMetValGluLeuVal	"
	"		"	AlaIleSerArgLysMetValGluLeu	"
	"		"	LysMetValGluLeuValHisPheLeuLeu	"
	"		"	LysMetValGluLeuValHisPheLeuLeuLeu	"
	"		"	LeuLeuLeuLysTyrArgAlaArgGluProVal	"
	"		"	LeuLeuLysTyrArgAlaArgGluProVal	"
	"		"	ValLeuArgAsnCysGlnAspPhePheProVal	"
	"		"	TyrLeuGlnLeuValPheGlyIleGluValVal	"
	"		"	GlyIleGluValValGluValValProIle	"
	"		"	ProIleSerHisLeuTyrIleLeuVal	"
	"		"	HisLeuTyrIleLeuValThrCysLeu	"
	"		"	HisLeuTyrIleLeuValThrCysLeuGlyLeu	"

	"		"	TyrIleLeuValThrCysLeuGlyLeu	"
	"		"	CysLeuGlyLeuSerTyrAspGlyLeu	"
	"		"	CysLeuGlyLeuSerTyrAspGlyLeuLeu	"
	"		"	ValMetProLysThrGlyLeuLeuIle	"
	"		"	ValMetProLysThrGlyLeuLeuIleIle	"
	"		"	ValMetProLysThrGlyLeuLeuIleIleVal	"
	"		"	GlyLeuLeuIleIleValLeuAlaIle	"
	"		"	GlyLeuLeuIleIleValLeuAlaIleIle	"
	"		"	GlyLeuLeuIleIleValLeuAlaIleIleAla	"
	"		"	LeuLeuIleIleValLeuAlaIleIle	"
	"		"	LeuLeuIleIleValLeuAlaIleIleAla	"
	"		"	LeuLeuIleIleValLeuAlaIleIleAlaIle	"
	"		"	LeuIleIleValLeuAlaIleIleAla	"
	"		"	LeuIleIleValLeuAlaIleIleAlaIle	"
	"		"	IleIleAlaIleGluGlyAspCysAla	"
	"		"	LysIleTrpGluGluLeuSerMetLeu	"
	"		"	LeuMetGlnAspLeuValGlnGluAsnTyrLeu	"
	"		"	PheLeuTrpGlyProArgAlaLeuIle	"
	"		"	LeuIleGluThrSerTyrValLysVal	"
	"		"	AlaLeuIleGluThrSerTyrValLysValLeu	"
	"		"	ThrLeuLysIleGlyGlyGluProHisIle	"
	"		"	HisIleSerTyrProProLeuHisGluArgAla	"
	"		"	GlnThrAlaSerSerSerSerThrLeu	"
	"		"	GlnThrAlaSerSerSerSerThrLeuVal	"

	"		"	ValThrLeuGlyGluValProAlaAla	"
	"		"	ValThrLysAlaGluMetLeuGluSerVal	"
	"		"	ValThrLysAlaGluMetLeuGluSerValLeu	"
	"		"	ValThrCysLeuGlyLeuSerTyrAspGlyLeu	"
	"		"	LysThrGlyLeuLeuIleIleValLeu	"
	"		"	LysThrGlyLeuLeuIleIleValLeuAla	"
	"		"	LysThrGlyLeuLeuIleIleValLeuAlaIle	"
	"		"	HisThrLeuLysIleGlyGlyGluProHisIle	"
	"		"	MetLeuAspLeuGlnProGluThrThr	"
	Mage-3 peptides		HLA-A2	GlyLeuGluAlaArgGlyGluAlaLeu	5,585,461
	"		"	AlaLeuSerArgLysValAlaGluLeu	"
	"		"	PheLeuTrpGlyProArgAlaLeuVal	"
	"		"	ThrLeuValGluValThrLeuGlyGluVal	"
	"		"	AlaLeuSerArgLysValAlaGluLeuVal	"
	"		"	AlaLeuValGluThrSerTyrValLysVal	"
	Tyrosinase		HLA-A2	TyrMetAsnGlyThrMetSerGlnVal	5,487,974
	"		"	MetLeuLeuAlaValLeuTyrCysLeuLeu	"
	Tyrosinase		HLA-A2	MetLeuLeuAlaValLeuTyrCysLeu	5,530,096
	"		"	LeuLeuAlaValLeuTyrCysLeuLeu	"
	Tyrosinase		HLA-A2 and HLA-B44	SerGluIleTrpArgAspIleAspPheAlaHisGluAla	5,519,117
	"		"	SerGluIleTrpArgAspIleAspPhe	"
	"		"	GluGluAsnLeuLeuAspPheValArgPhe	"
	Melan			EAAGIGILTV	Jäger, E. et al.

	A/MART-1				Granulocyte-macrophage-colony-stimulating Factor Enhances Immune Responses To Melanoma-'associated Peptides in vivo Int. J Cancer 67, 54-62 (1996)
	Tyrosinase			MLLAVLYCL	"
	"			YMDGTMSQV	"
	gp100/Pmel 17			YLEPGPVTA	"
	"			LLDGTATLRL	"
	Influenza matrix			GILGFVFTL	"
	MAGE-1			EADPTGHSY	"
	MAGE-1		HLA-A1	EADPTGHSY	DIRECTLY FROM DAVID'S LIST
	BAGE		HLA-C	MAARAVFLALSAQL LQARLMKE	"
	"		"	MAARAVFLALSAQL LQ	"
	"		"	AARAVFLAL	"
Influenza	PR8 NP	147-154	K^d	IYQRIRALV	Falk et al., Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules
SELF PEPTIDE	P815		"	SYFPEITHI	"
Influenza	Jap HA 523-549		"	IYATVAGSL	"
"	"		"	VYQILAIYA	"
"	"		"	IYSTVASSL	"
"	JAP HA 202-221		"	LYQNVGTYV	"
	HLA-A24		"	RYLENQKRT	"
	HLA-Cw3		"	RYLKNGKET	"
	P815		"	KYQAVTTTL	"

Plasmodium berghen	CSP		"	SYIPSAEKI	"
Plasmodium yoelli	CSP		"	SYVPSAFQI	"
Vesicular stomatitis viruse	NP 52-59		K^b	RGYVYQGL	"
Ovalbumin			"	SIINFEKL	"
Sandal Virus	NP 321-332		"	APGNYPAL	"
				VPYGSFKHV	Morel et al., Processing of some antigens by the standard proteasome but not by the immunoproteasome results in poor presentation by dendritic cells, Immunity, vol. 12:107-117, 2000.

			MOTIFS		
influenza	PR8 NP		K^d restricted peptide motif	TYQRTRALV	5,747,269
self peptide	P815		"	SYFPEITHI	"
influenza	JAP HA		"	IYATVAGSL	"
influenza	JAP HA		"	VYQILAIYA	"
influenza	PR8 HA		"	IYSTVASSL	"
influenza	JAP HA		"	LYQNVGTYV	"
			HLA-A24	RYLENGKETL	"
			HLA-Cw3	RYLKNGKETL	"
	P815 tumour antigen		"	KYQAVTTTL	"
Plasmodium berghei	CSP		"	SYIPSAEKI	"
Plasmodium yoeli	CSP		"	SYVPSAEQI	"
influenza	NP		D^b - restricted peptide motif	ASNENMETM	"
adenovirus	E1A		"	SGPSNTPPEI	"
lymphocytic choriomeningitis			"	SGVENPGGYCL	"
simian virus	40 T		"	SAINNY . . .	"
HIV	reverse transcriptase		HLA-A2.1-restricted peptide motif	ILKEPVHGV	"
	influenza matrix protein		"	GILGFVFTL	"
influenza	influenza matrix protein		"	ILGFVFTLTV	"
HIV	Gag protein			FLQSRPEPT	"
HIV	Gag protein			AMQMLKE . .	"
HIV	Gag protein			PIAPGQMRE	"
HIV	Gag protein			QMKDCTERQ	"
			HLA-A*0205-restricted peptide motif	VYGVIQK	"

Table 5

VSV-NP peptide (49-62)
LCMV-NP peptide (118-132)
LCMV glycoprotein peptide. 33-41
ISNQLTLDSNTKYFHKLN
ISNQLTLDSNTKYFHKL
ISNQLTLDSNTKYFHK
VDTFLEDVKNLYHSEA
KPRAIVVDPVHGFMY
KQTISPDYRNMI
Y[DFIMDPKEKDKV
NIQLINDQEVARFD
LLSFVRDLNQYRADI
LPKPPKPVSKMRMATPL
LPKPPKPVSKMRMATPLLMQALP
LPKPPKPVSKMRMATPLLMQALPM
PKPPKPVSKMRMATPL
PKPPKPVSKMRMATPLLMQA
KPPKPVSKMRMATPLLMQ
KPPKPVSKMRMATPLLMQALPM
VDDTQFVRFDSDAASQ
ATKYGNMTEDHVMHLLQNA
VFLLLLADKVPETSLS
LNKILLDEQAQWK
GPPKLDIRKEEKQIMIDIFH
GPPKLDIRKEEKQIMIDIFHP
GFKAIRPDKKSNPIIRTV
YANILLDRRVPQTDMTF
NLFLKSDGRIKYTLNKNSLK
IPDNLFLKSDGRIKYTLNKN
IPDNLFLKSDGRIKYTLNK
IPDNLFLKSDGRIKYTLN
IPDNLFLKSDGRIKYTL
NLFLKSDGRIKYTLNK
NLFLKSDGRIKYTLN
VTTLNSDLKYNALDLTN
VGSDWRFLRGYHQYA

[0078] Still further embodiments are directed to methods, uses, therapies and compositions related to epitopes with specificity for MHC, including, for example, those listed in Tables 6-10. Other embodiments include one or more of the MHCs listed in Tables 6-10, including combinations of the same, while other embodiments specifically exclude any one or more of the MHCs or combinations thereof. Tables 8-10 include frequencies for the listed HLA antigens.

Table 6

Class I MHC Molecules

Class I

Human

HLA-A1
HLA-A*0101
HLA-A*0201
HLA-A*0202
HLA-A*0203
HLA-A*0204
HLA-A*0205
HLA-A*0206
HLA-A*0207
HLA-A*0209
HLA-A*0214
HLA-A3
HLA-A*0301
HLA-A*1101
HLA-A23
HLA-A24
HLA-A25
HLA-A*2902
HLA-A*3101
HLA-A*3302
HLA-A*6801
HLA-A*6901
HLA-B7
HLA-B*0702
HLA-B*0703
HLA-B*0704
HLA-B*0705
HLA-B8
HLA-B13
HLA-B14
HLA-B*1501 (B62)
HLA-B17
HLA-B18
HLA-B22
HLA-B27
HLA-B*2702
HLA-B*2704
HLA-B*2705
HLA-B*2709
HLA-B35
HLA-B*3501
HLA-B*3502
HLA-B*3701
HLA-B*3801
HLA-B*39011
HLA-B*3902

HLA-B40
HLA-B*40012 (B60)
HLA-B*4006 (B61)
HLA-B44
HLA-B*4402
HLA-B*4403
HLA-B*4501
HLA-B*4601
HLA-B51
HLA-B*5101
HLA-B*5102
HLA-B*5103
HLA-B*5201
HLA-B*5301
HLA-B*5401
HLA-B*5501
HLA-B*5502
HLA-B*5601
HLA-B*5801
HLA-B*6701
HLA-B*7301
HLA-B*7801
HLA-Cw*0102
HLA-Cw*0301
HLA-Cw*0304
HLA-Cw*0401
HLA-Cw*0601
HLA-Cw*0602
HLA-Cw*0702
HLA-Cw8
HLA-Cw*1601 M
HLA-G

Murine

H2-K^d
H2-D^d
H2-L^d
H2-K^b
H2-D^b
H2-K^k
H2-K^{kml}
Qa-1^a
Qa-2
H2-M3

Rat

RT1.A^a
RT1.A^l

Bovine
Bota-A11
Bota-A20

Chicken
B-F4
B-F12
B-F15
B-F19

Chimpanzee
Patr-A*04
Patr-A*11
Patr-B*01
Patr-B*13
Patr-B*16

Baboon
Papa-A*06

Macaque
Mamu-A*01

Swine
SLA (haplotype d/d)

Virus homolog
hCMV class I homolog UL18

Table 7

Class I MHC Molecules

Class I

Human
HLA-A1
HLA-A*0101
HLA-A*0201
HLA-A*0202
HLA-A*0204
HLA-A*0205
HLA-A*0206
HLA-A*0207
HLA-A*0214
HLA-A3
HLA-A*1101
HLA-A24
HLA-A*2902
HLA-A*3101
HLA-A*3302
HLA-A*6801
HLA-A*6901

HLA-B7
HLA-B*0702
HLA-B*0703
HLA-B*0704
HLA-B*0705
HLA-B8
HLA-B14
HLA-B*1501 (B62)
HLA-B27
HLA-B*2702
HLA-B*2705
HLA-B35
HLA-B*3501
HLA-B*3502
HLA-B*3701
HLA-B*3801
HLA-B*39011
HLA-B*3902
HLA-B40
HLA-B*40012 (B60)
HLA-B*4006 (B61)
HLA-B44
HLA-B*4402
HLA-B*4403
HLA-B*4601
HLA-B51
HLA-B*5101
HLA-B*5102
HLA-B*5103
HLA-B*5201
HLA-B*5301
HLA-B*5401
HLA-B*5501
HLA-B*5502
HLA-B*5601
HLA-B*5801
HLA-B*6701
HLA-B*7301
HLA-B*7801
HLA-Cw*0102
HLA-Cw*0301
HLA-Cw*0304
HLA-Cw*0401
HLA-Cw*0601
HLA-Cw*0602
HLA-Cw*0702
HLA-G

Murine
$H2\text{-}K^d$

H2-D^d
H2-L^d
H2-K^b
H2-D^b
H2-K^k
H2-K^{kml}
Qa-2

Rat
RT1.A^a
RT1.A^l

Bovine
Bota-A11
Bota-A20

Chicken
B-F4
B-F12
B-F15
B-F19

Virus homolog
hCMV class I homolog UL18

Table 8

[0079] Estimated gene frequencies of HLA-A antigens

Antigen	CAU		AFR		ASI		LAT		NAT	
	Gf[a]	SE[b]	Gf	SE	Gf	SE	Gf	SE	Gf	SE
A1	15.1843	0.0489	5.7256	0.0771	4.4818	0.0846	7.4007	0.0978	12.0316	0.2533
A2	28.6535	0.0619	18.8849	0.1317	24.6352	0.1794	28.1198	0.1700	29.3408	0.3585
A3	13.3890	0.0463	8.4406	0.0925	2.6454	0.0655	8.0789	0.1019	11.0293	0.2437
A28	4.4652	0.0280	9.9269	0.0997	1.7657	0.0537	8.9446	0.1067	5.3856	0.1750
A36	0.0221	0.0020	1.8836	0.0448	0.0148	0.0049	0.1584	0.0148	0.1545	0.0303
A23	1.8287	0.0181	10.2086	0.1010	0.3256	0.0231	2.9269	0.0628	1.9903	0.1080
A24	9.3251	0.0395	2.9668	0.0560	22.0391	0.1722	13.2610	0.1271	12.6613	0.2590
A9 unsplit	0.0809	0.0038	0.0367	0.0063	0.0858	0.0119	0.0537	0.0086	0.0356	0.0145
A9 total	11.2347	0.0429	13.2121	0.1128	22.4505	0.1733	16.2416	0.1382	14.6872	0.2756
A25	2.1157	0.0195	0.4329	0.0216	0.0990	0.0128	1.1937	0.0404	1.4520	0.0924
A26	3.8795	0.0262	2.8284	0.0547	4.6628	0.0862	3.2612	0.0662	2.4292	0.1191
A34	0.1508	0.0052	3.5228	0.0610	1.3529	0.0470	0.4928	0.0260	0.3150	0.0432
A43	0.0018	0.0006	0.0334	0.0060	0.0231	0.0062	0.0055	0.0028	0.0059	0.0059
A66	0.0173	0.0018	0.2233	0.0155	0.0478	0.0089	0.0399	0.0074	0.0534	0.0178
A10 unsplit	0.0790	0.0038	0.0939	0.0101	0.1255	0.0144	0.0647	0.0094	0.0298	0.0133
A10 total	6.2441	0.0328	7.1348	0.0850	6.3111	0.0993	5.0578	0.0816	4.2853	0.1565
A29	3.5796	0.0252	3.2071	0.0582	1.1233	0.0429	4.5156	0.0774	3.4345	0.1410
A30	2.5067	0.0212	13.0969	0.1129	2.2025	0.0598	4.4873	0.0772	2.5314	0.1215
A31	2.7386	0.0221	1.6556	0.0420	3.6005	0.0761	4.8328	0.0800	6.0881	0.1855
A32	3.6956	0.0256	1.5384	0.0405	1.0331	0.0411	2.7064	0.0604	2.5521	0.1220
A33	1.2080	0.0148	6.5607	0.0822	9.2701	0.1191	2.6593	0.0599	1.0754	0.0796
A74	0.0277	0.0022	1.9949	0.0461	0.0561	0.0096	0.2027	0.0167	0.1068	0.0252
A19 unsplit	0.0567	0.0032	0.2057	0.0149	0.0990	0.0128	0.1211	0.0129	0.0475	0.0168
A19 total	13.8129	0.0468	28.2593	0.1504	17.3846	0.1555	19.5252	0.1481	15.8358	0.2832
AX	0.8204	0.0297	4.9506	0.0963	2.9916	0.1177	1.6332	0.0878	1.8454	0.1925

[a]Gene frequency.
[b]Standard error.

Table 9

Estimated gene frequencies for HLA-B antigens

Antigen	CAU		AFR		ASI		LAT		NAT	
	Gf[a]	SE[b]	Gf	SE	Gf	SE	Gf	SE	Gf	SE
B7	12.1782	0.0445	10.5960	0.1024	4.2691	0.0827	6.4477	0.0918	10.9845	0.2432
B8	9.4077	0.0397	3.8315	0.0634	1.3322	0.0467	3.8225	0.0715	8.5789	0.2176
B13	2.3061	0.0203	0.8103	0.0295	4.9222	0.0886	1.2699	0.0416	1.7495	0.1013
B14	4.3481	0.0277	3.0331	0.0566	0.5004	0.0287	5.4166	0.0846	2.9823	0.1316
B18	4.7980	0.0290	3.2057	0.0582	1.1246	0.0429	4.2349	0.0752	3.3422	0.1391
B27	4.3831	0.0278	1.2918	0.0372	2.2355	0.0603	2.3724	0.0567	5.1970	0.1721
B35	9.6614	0.0402	8.5172	0.0927	8.1203	0.1122	14.6516	0.1329	10.1198	0.2345
B37	1.4032	0.0159	0.5916	0.0252	1.2327	0.0449	0.7807	0.0327	0.9755	0.0759
B41	0.9211	0.0129	0.8183	0.0296	0.1303	0.0147	1.2818	0.0418	0.4766	0.0531
B42	0.0608	0.0033	5.6991	0.0768	0.0841	0.0118	0.5866	0.0284	0.2856	0.0411
B46	0.0099	0.0013	0.0151	0.0040	4.9292	0.0886	0.0234	0.0057	0.0238	0.0119
B47	0.2069	0.0061	0.1305	0.0119	0.0956	0.0126	0.1832	0.0159	0.2139	0.0356
B48	0.0865	0.0040	0.1316	0.0119	2.0276	0.0575	1.5915	0.0466	1.0267	0.0778
B53	0.4620	0.0092	10.9529	0.1039	0.4315	0.0266	1.6982	0.0481	1.0804	0.0798

Antigen	CAU		AFR		ASI		LAT		NAT	
	Gf[a]	SE[b]	Gf	SE	Gf	SE	Gf	SE	Gf	SE
B59	0.0020	0.0006	0.0032	0.0019	0.4277	0.0265	0.0055	0.0028	0[c]	
B67	0.0040	0.0009	0.0086	0.0030	0.2276	0.0194	0.0055	0.0028	0.0059	0.0059
B70	0.3270	0.0077	7.3571	0.0866	0.8901	0.0382	1.9266	0.0512	0.6901	0.0639
B73	0.0108	0.0014	0.0032	0.0019	0.0132	0.0047	0.0261	0.0060	0[c]	
B51	5.4215	0.0307	2.5980	0.0525	7.4751	0.1080	6.8147	0.0943	6.9077	0.1968
B52	0.9658	0.0132	1.3712	0.0383	3.5121	0.0752	2.2447	0.0552	0.6960	0.0641
B5 unsplit	0.1565	0.0053	0.1522	0.0128	0.1288	0.0146	0.1546	0.0146	0.1307	0.0278
B5 total	6.5438	0.0435	4.1214	0.0747	11.1160	0.1504	9.2141	0.1324	7.7344	0.2784
B44	13.4838	0.0465	7.0137	0.0847	5.6807	0.0948	9.9253	0.1121	11.8024	0.2511
B45	0.5771	0.0102	4.8069	0.0708	0.1816	0.0173	1.8812	0.0506	0.7603	0.0670
B12 unsplit	0.0788	0.0038	0.0280	0.0055	0.0049	0.0029	0.0193	0.0051	0.0654	0.0197
B12 total	14.1440	0.0474	11.8486	0.1072	5.8673	0.0963	11.8258	0.1210	12.6281	0.2584
B62	5.9117	0.0320	1.5267	0.0404	9.2249	0.1190	4.1825	0.0747	6.9421	0.1973
B63	0.4302	0.0088	1.8865	0.0448	0.4438	0.0270	0.8083	0.0333	0.3738	0.0471
B75	0.0104	0.0014	0.0226	0.0049	1.9673	0.0566	0.1101	0.0123	0.0356	0.0145
B76	0.0026	0.0007	0.0065	0.0026	0.0874	0.0120	0.0055	0.0028	0	
B77	0.0057	0.0010	0.0119	0.0036	0.0577	0.0098	0.0083	0.0034	0[c]	0.0059
B15 unsplit	0.1305	0.0049	0.0691	0.0086	0.4301	0.0266	0.1820	0.0158	0.0059	0.0206
B15 total	6.4910	0.0334	3.5232	0.0608	12.2112	0.1344	5.2967	0.0835	0.0715	0.2035
									7.4290	
B38	2.4413	0.0209	0.3323	0.0189	3.2818	0.0728	1.9652	0.0517	1.1017	0.0806
B39	1.9614	0.0188	1.2893	0.0371	2.0352	0.0576	6.3040	0.0909	4.5527	0.1615
B16 unsplit	0.0638	0.0034	0.0237	0.0051	0.0644	0.0103	0.1226	0.0130	0.0593	0.0188
B16 total	4.4667	0.0280	1.6453	0.0419	5.3814	0.0921	8.3917	0.1036	5.7137	0.1797
B57	3.5955	0.0252	5.6746	0.0766	2.5782	0.0647	2.1800	0.0544	2.7265	0.1260
B58	0.7152	0.0114	5.9546	0.0784	4.0189	0.0803	1.2481	0.0413	0.9398	0.0745
B17 unsplit	0.2845	0.0072	0.3248	0.0187	0.3751	0.0248	0.1446	0.0141	0.2674	0.0398
B17 total	4.5952	0.0284	11.9540	0.1076	6.9722	0.1041	3.5727	0.0691	3.9338	0.1503
B49	1.6452	0.0172	2.6286	0.0528	0.2440	0.0200	2.3353	0.0562	1.5462	0.0953
B50	1.0580	0.0138	0.8636	0.0304	0.4421	0.0270	1.8883	0.0507	0.7862	0.0681
B21 unsplit	0.0702	0.0036	0.0270	0.0054	0.0132	0.0047	0.0771	0.0103	0.0356	0.0145
B21 total	2.7733	0.0222	3.5192	0.0608	0.6993	0.0339	4.3007	0.0755	2.3680	0.1174
B54	0.0124	0.0015	0.0183	0.0044	2.6873	0.0660	0.0289	0.0063	0.0534	0.0178
B55	1.9046	0.0185	0.4895	0.0229	2.2444	0.0604	0.9515	0.0361	1.4054	0.0909
B56	0.5527	0.0100	0.2686	0.0170	0.8260	0.0368	0.3596	0.0222	0.3387	0.0448
B22 unsplit	0.1682	0.0055	0.0496	0.0073	0.2730	0.0212	0.0372	0.0071	0.1246	0.0272
B22 total	2.0852	0.0217	0.8261	0.0297	6.0307	0.0971	1.3771	0.0433	1.9221	0.1060
B60	5.2222	0.0302	1.5299	0.0404	8.3254	0.1135	2.2538	0.0553	5.7218	0.1801
B61	1.1916	0.0147	0.4709	0.0225	6.2072	0.0989	4.6691	0.0788	2.6023	0.1231
B40 unsplit	0.2696	0.0070	0.0388	0.0065	0.3205	0.0230	0.2473	0.0184	0.2271	0.0367
B40 total	6.6834	0.0338	2.0396	0.0465	14.8531	0.1462	7.1702	0.0963	8.5512	0.2168
BX	1.0922	0.0252	3.5258	0.0802	3.8749	0.0988	2.5266	0.0807	1.9867	0.1634

[a]Gene frequency. [b]Standard error. [c]The observed gene count was zero.

Table 10.
Estimated gene frequencies of HLA-DR antigens

Antigen	CAU		AFR		ASI		LAT		NAT	
	Gf[a]	SE[b]	Gf	SE	Gf	SE	Gf	SE	Gf	SE
DR1	10.2279	0.0413	6.8200	0.0832	3.4628	0.0747	7.9859	0.1013	8.2512	0.2139
DR2	15.2408	0.0491	16.2373	0.1222	18.6162	0.1608	11.2389	0.1182	15.3932	0.2818
DR3	10.8708	0.0424	13.3080	0.1124	4.7223	0.0867	7.8998	0.1008	10.2549	0.2361
DR4	16.7589	0.0511	5.7084	0.0765	15.4623	0.1490	20.5373	0.1520	19.8264	0.3123
DR6	14.3937	0.0479	18.6117	0.1291	13.4471	0.1404	17.0265	0.1411	14.8021	0.2772
DR7	13.2807	0.0463	10.1317	0.0997	6.9270	0.1040	10.6726	0.1155	10.4219	0.2378
DR8	2.8820	0.0227	6.2673	0.0800	6.5413	0.1013	9.7731	0.1110	6.0059	0.1844
DR9	1.0616	0.0139	2.9646	0.0559	9.7527	0.1218	1.0712	0.0383	2.8662	0.1291
DR10	1.4790	0.0163	2.0397	0.0465	2.2304	0.0602	1.8044	0.0495	1.0896	0.0801
DR11	9.3180	0.0396	10.6151	0.1018	4.7375	0.0869	7.0411	0.0955	5.3152	0.1740
DR12	1.9070	0.0185	4.1152	0.0655	10.1365	0.1239	1.7244	0.0484	2.0132	0.1086
DR5 unsplit	1.2199	0.0149	2.2957	0.0493	1.4118	0.0480	1.8225	0.0498	1.6769	0.0992
DR5 total	12.4449	0.0045	17.0260	0.1243	16.2858	0.1516	10.5880	0.1148	9.0052	0.2218
DRX	1.3598	0.0342	0.8853	0.0760	2.5521	0.1089	1.4023	0.0930	2.0834	0.2037

[a]Gene frequency.
[b]Standard error.

[0080] It can be desirable to express housekeeping peptides in the context of a larger protein. Processing can be detected even when a small number of amino acids are present beyond the terminus of an epitope. Small peptide hormones are usually proteolytically processed from longer translation products, often in the size range of approximately 60-120 amino acids. This fact has led some to assume that this is the minimum size that can be efficiently translated. In some embodiments, the housekeeping peptide can be embedded in a translation product of at least about 60 amino acids, in others 70, 80, 90 amino acids, and in still others 100, 110 or 120 amino acids, for example. In other embodiments the housekeeping peptide can be embedded in a translation product of at least about 50, 30, or 15 amino acids.

[0081] Due to differential proteasomal processing, the immunoproteasome of the pAPC produces peptides that are different from those produced by the housekeeping proteasome in peripheral body cells. Thus, in expressing a housekeeping peptide in the context of a larger protein, it is preferably expressed in the pAPC in a context other than its full-length native sequence, because, as a housekeeping epitope, it is generally only efficiently processed from the native protein by the housekeeping proteasome, which is not active in the pAPC. In order to encode the housekeeping epitope in a DNA sequence encoding a larger polypeptide, it is useful to find flanking areas on either side of the sequence encoding the epitope that permit appropriate cleavage by the immunoproteasome in order to liberate that housekeeping epitope. Such a sequence promoting

appropriate processing is referred to hereinafter as having substrate or liberation sequence function. Altering flanking amino acid residues at the N-terminus and C-terminus of the desired housekeeping epitope can facilitate appropriate cleavage and generation of the housekeeping epitope in the pAPC. Sequences embedding housekeeping epitopes can be designed *de novo* and screened to determine which can be successfully processed by immunoproteasomes to liberate housekeeping epitopes.

[0082] Alternatively, another strategy is very effective for identifying sequences allowing production of housekeeping epitopes in APC. A contiguous sequence of amino acids can be generated from head to tail arrangement of one or more housekeeping epitopes. A construct expressing this sequence is used to immunize an animal, and the resulting T cell response is evaluated to determine its specificity to one or more of the epitopes in the array. These immune responses indicate housekeeping epitopes that are processed in the pAPC effectively. The necessary flanking areas around this epitope are thereby defined. The use of flanking regions of about 4-6 amino acids on either side of the desired peptide can provide the necessary information to facilitate proteasome processing of the housekeeping epitope by the immunoproteasome. Therefore, a substrate or liberation sequence of approximately 16-22 amino acids can be inserted into, or fused to, any protein sequence effectively to result in that housekeeping epitope being produced in an APC. In some embodiments, a broader context of a substrate sequence can also influence processing. In such embodiments, comparisons of a liberaton sequence in a variety of contexts can be useful in further optimizing a particular substrate sequence. In alternate embodiments the whole head-to-tail array of epitopes, or just the epitopes immediately adjacent to the correctly processed housekeeping epitope can be similarly transferred from a test construct to a vaccine vector.

[0083] In a preferred embodiment, the housekeeping epitopes can be embedded between known immune epitopes, or segments of such, thereby providing an appropriate context for processing. The abutment of housekeeping and immune epitopes can generate the necessary context to enable the immunoproteasome to liberate the housekeeping epitope, or a larger fragment, preferably including a correct C-terminus. It can be useful to screen constructs to verify that the desired epitope is produced. The abutment of housekeeping epitopes can generate a site cleavable by the immunoproteasome. Some embodiments of the invention employ known epitopes to flank housekeeping epitopes in test substrates; in others, screening as described below is used, whether the flanking regions are arbitrary sequences or mutants of the natural flanking sequence, and whether or not knowledge of proteasomal cleavage preferences are used in designing the substrates.

[0084] Cleavage at the mature N-terminus of the epitope, while advantageous, is not required, since a variety of N-terminal trimming activities exist in the cell that can generate the

mature N-terminus of the epitope subsequent to proteasomal processing. It is preferred that such N-terminal extension be less than about 25 amino acids in length and it is further preferred that the extension have few or no proline residues. Preferably, in screening, consideration is given not only to cleavage at the ends of the epitope (or at least at its C-terminus), but consideration also can be given to ensure limited cleavage within the epitope.

[0085] Shotgun approaches can be used in designing test substrates and can increase the efficiency of screening. In one embodiment multiple epitopes can be assembled one after the other, with individual epitopes possibly appearing more than once. The substrate can be screened to determine which epitopes can be produced. In the case where a particular epitope is of concern, a substrate can be designed in which it appears in multiple different contexts. When a single epitope appearing in more than one context is liberated from the substrate additional secondary test substrates, in which individual instances of the epitope are removed, disabled, or are unique, can be used to determine which are being liberated and truly confer substrate or liberation sequence function.

[0086] Several readily practicable screens exist. A preferred *in vitro* screen utilizes proteasomal digestion analysis, using purified immunoproteasomes, to determine if the desired housekeeping epitope can be liberated from a synthetic peptide embodying the sequence in question. The position of the cleavages obtained can be determined by techniques such as mass spectrometry, HPLC, and N-terminal pool sequencing; as described in greater detail in U.S. Patent Application Nos. 09/561,074, 09/560,465 and 10/117,937, and Provisional U.S. Patent Application Nos. 60/282,211, 60/337,017, and 60/363, 210.

[0087] Alternatively, *in vivo* and cell-based screens such as immunization or target sensitization can be employed. For immunization a nucleic acid construct capable of expressing the sequence in question is used. Harvested CTL can be tested for their ability to recognize target cells presenting the housekeeping epitope in question. Such targets cells are most readily obtained by pulsing cells expressing the appropriate MHC molecule with synthetic peptide embodying the mature housekeeping epitope. Alternatively, immunization can be carried out using cells known to express housekeeping proteasome and the antigen from which the housekeeping epitope is derived, either endogenously or through genetic engineering. To use target sensitization as a screen, CTL, or preferably a CTL clone, that recognizes the housekeeping epitope can be used. In this case it is the target cell that expresses the embedded housekeeping epitope (instead of the pAPC during immunization) and it must express immunoproteasome. Generally, the cell or target cell can be transformed with an appropriate nucleic acid construct to confer expression of the embedded

housekeeping epitope. Loading with a synthetic peptide embodying the embedded epitope using peptide loaded liposomes, or complexed with cationic lipid protein transfer reagents such as BIOPORTER™ (Gene Therapy Systems, San Diego, CA), represents an alternative.

[0088] Once sequences with substrate or liberation sequence function are identified they can be encoded in nucleic acid vectors, chemically synthesized, or produced recombinantly. In any of these forms they can be incorporated into immunogenic compositions. Such compositions can be used *in vitro* in vaccine development or in the generation or expansion of CTL to be used in adoptive immunotherapy. *In vivo* they can be used to induce, amplify or sustain and active immune response. The uptake of polypeptides for processing and presentation can be greatly enhanced by packaging with cationic lipid, the addition of a tract of cationic amino acids such as poly-L-lysine (Ryser, H.J. et al., *J. Cell Physiol.* 113:167-178, 1982; Shen, W.C. & Ryser, H.J., *Proc. Natl. Aced. Sci. USA* 75:1872-1876, 1978), the incorporation into branched structures with importation signals (Sheldon, K. et al., *Proc. Natl. Aced. Sci. USA* 92:2056-2060, 1995), or mixture with or fusion to polypeptides with protein transfer function including peptide carriers such as pep-1 (Morris, M.C., et al., *Nat. Biotech.* 19:1173-1176, 2001), the PreS2 translocation motif of hepatitis B virus surface antigen, VP22 of herpes viruses, and HIV-TAT protein (Oess, S. & Hildt, E., *Gene Ther.* 7:750-758, 2000; Ford, K.G., et al., *Gene Ther.* 8:1-4, 2001; Hung, C.F. et al., *J. Virol.* 76:2676-2682, 2002; Oliveira, S.C., et a;. *Hum. Gene Ther.* 12:1353-1359, 2001; Normand, N. et al., *J. Biol. Chem.* 276:15042-15050, 2001; Schwartz, J.J. & Zhang, S., *Curr. Opin. Mol. Ther.* 2:162-167, 2000; Elliot G., 7 Hare, P. Cell 88:223-233, 1997), among other methodologies. Particularly for fusion proteins the immunogen can be produced in culture and the purified protein administered or, in the alternative, the nucleic acid vector can be administered so that the immunogen is produced and secreted by cells transformed *in vivo*. In either scenario the transport function of the fusion protein facilitates uptake by pAPC.

EXAMPLES

Example 1

[0089] A recombinant DNA plasmid vaccine, pMA2M, which encodes one polypeptide with an HLA A2-specific CTL epitope ELAGIGILTV (SEQ ID NO. 1) from melan-A (26-35A27L), and a portion (amino acids 31-96) of melan-A (SEQ ID NO. 2) including the epitope clusters at amino acids 31-48 and 56-69, was constructed. These clusters were previously disclosed in U.S. Patent Application No. 09/561,571 entitled EPITOPE CLUSTERS. Flanking the defined melan-A CTL epitope are short amino acid sequences derived from human tyrosinase (SEQ ID NO. 3) to facilitate liberation of the melan-A housekeeping epitope by processing by the immunoproteasome.

In addition, these amino acid sequences represent potential CTL epitopes themselves. The cDNA sequence for the polypeptide in the plasmid is under the control of promoter/enhancer sequence from cytomegalovirus (CMVp) (see Figure 1), which allows efficient transcription of messenger for the polypeptide upon uptake by APCs. The bovine growth hormone polyadenylation signal (BGH polyA) at the 3' end of the encoding sequence provides a signal for polyadenylation of the messenger to increase its stability as well as for translocation out of nucleus into the cytoplasm for translation. To facilitate plasmid transport into the nucleus after uptake, a nuclear import sequence (NIS) from simian virus 40 (SV40) has been inserted in the plasmid backbone. The plasmid carries two copies of a CpG immunostimulatory motif, one in the NIS sequence and one in the plasmid backbone. Lastly, two prokaryotic genetic elements in the plasmid are responsible for amplification in *E.coli*, the kanamycin resistance gene (Kan R) and the pMB1 bacterial origin of replication.

SUBSTRATE or LIBERATION sequence

[0090] The amino acid sequence of the encoded polypeptide (94 amino acid residues in length) (SEQ ID NO. 4) containing a 28 amino acid substrate or liberation sequence at its N-terminus (SEQ ID NO. 5) is given below:

[0091] MLLAVLYCL-ELAGIGILTV-YMDGTMSQV-GILTVILGVLLLIGCWYCRRRNGYRALMDKSLHVGTQCALTRRCPQEGFDHRDSKVSLQEK NCEPV

[0092] The first 9 amino acid residues are derived from tyrosinase$_{1-9}$ (SEQ ID NO. 6), the next ten constitute melan-A (26-35A27L) (SEQ ID NO. 1), and amino acid residues 20 to 29 are derived from tyrosinase$_{369-377}$ (SEQ ID NO. 7). These two tyrosinase nonamer sequences both represent potential HLA A2-specific CTL epitopes. Amino acid residues 10-19 constitute melan-A (26-35A27L) an analog of an HLA A2-specific CTL epitope from melan-A, EAAGIGILTV (SEQ ID NO. 8), with an elevated potency in inducing CTL responses during *in vitro* immunization of human PBMC and *in vivo* immunization in mice. The segment of melan-A constituting the rest of the polypeptide (amino acid residues 30 to 94) contain a number of predicted HLA A2-specific epitopes, including the epitope clusters cited above, and thus can be useful in generating a response to immune epitopes as described at length in the patent applications 'Epitope Synchronization in Antigen Presenting Cells' and 'Epitope Clusters'. This region was also included to overcome any difficulties

that can be associated with the expression of shorter sequences. A drawing of pMA2M is shown in Figure 1.

Plasmid construction

[0093] A pair of long complementary oligonucleotides was synthesized which encoded the first 30 amino acid residues. In addition, upon annealing, these oligonucleotides generated the cohensive ends of *Afl* II at the 5' end and that of *EcoR* I at the 3' end. The melan A_{31-96} region was amplified with PCR using oligonucleotides carrying restriction sites for *EcoR* I at the 5' end and *Not* I at the 3' end. The PCR product was digested with *EcoR* I and *Not* I and ligated into the vector backbone, described in Example 1, that had been digested with *Afl* II and *Not* I, along with the annealed oligonucleotides encoding the amino terminal region in a three-fragment ligation. The entire coding sequence was verified by DNA sequencing. The sequence of the entire insert, from the *Afl* II site at the 5' end to the *Not* I site at the 3' end is disclosed as SEQ ID NO. 9. Nucleotides 12-293 encode the polypeptide.

Example 2

[0094] Three vectors containing melan-A (26-35A27L) (SEQ ID NO. 1) as an embedded housekeeping epitope were tested for their ability to induce a CTL response to this epitope in HLA-A2 transgenic HHD mice (Pascolo et al. *J. Exp. Med.* 185:2043-2051, 1997). One of the vectors was pMA2M described above (called pVAXM3 in Figure 3). In pVAXM2 the same basic group of 3 epitopes was repeated several times with the flanking epitopes truncated by differing degrees in the various repeats of the array. Specifically the cassette consisted of:

[0095] M-Tyr(5-9)-ELA-Tyr(369-373)-Tyr(4-9)-ELA-Tyr(369-374)-Tyr(3-9)-ELA-Tyr(369-375)-Tyr(2-9)-ELA

(SEQ ID NO. 10)

[0096] where ELA represents melan-A (26-35A27L) (SEQ ID NO. 1). This cassette was inserted in the same plasmid backbone as used for pVAXM3. The third, pVAXM1 is identical to pVAXM2 except that the epitope array is followed by an IRES (internal ribosome entry site for encephalomyocarditis virus) linked to a reading frame encoding melan-A 31-70.

[0097] Four groups of three HHD A2.1 mice were injected intranodally in surgically exposed inguinal lymph nodes with 25 µl of 1 mg/ml plasmid DNA in PBS on days 0, 3, and 6, each group receiving one of the three vectors or PBS alone. On day 14 the spleens were harvested and restimulated *in vitro* one time with 3-day LPS blasts pulsed with peptide (melan-A (26-

35A27L)(SEQ ID NO. 1)). The *in vitro* cultures were supplemented with Rat T-Stim (Collaborative Biomedical Products) on the 3rd day and assayed for cytolytic activity on the 7th day using a standard ^{51}Cr-release assay. Figures 2 to 5 show % specific lysis obtained using the cells immunized with PBS, pVAXM1, pVAXM2, and pVAXM3, respectively on T2 target cells and T2 target cells pulsed with melan-A (26-35A27L) (ELA) (SEQ ID NO. 1). All three vectors generated strong CTL responses. These data indicated that the plasmids have been taken up by APCs, the encoded polypeptide has been synthesized and proteolytically processed to produce the decamer epitope in question (that is, it had substrate or liberation sequence function), and that the epitope became HLA-A2 bound for presentation. Also, an isolated variant of pVAXM2, that terminates after the 55th amino acid, worked similarly well as the full length version (data not shown). Whether other potential epitopes within the expression cassette can also be produced and be active in inducing CTL responses can be determined by testing for CTL activity against target cells pulsed with corresponding synthetic peptides.

Example 3

An NY-ESO-1 (SEQ ID NO. 11) SUBSTRATE/LIBERATION Sequence

[0098] Six other epitope arrays were tested leading to the identification of a substrate/liberation sequence for the housekeeping epitope NY-ESO-$1_{157-165}$ (SEQ ID NO. 12). The component epitopes of the arrays were:

[0099]	SSX-2_{41-49}:	KASEKIFYV	(SEQ ID NO. 13)	Array element A
[0100]	NY-ESO-$1_{157-165}$:	SLLMWITQC	(SEQ ID NO. 12)	Array element B
[0101]	NY-ESO-$1_{163-171}$:	TQCFLPVFL	(SEQ ID NO. 14)	Array element C
[0102]	$PSMA_{288-297}$:	GLPSIPVHPI	(SEQ ID NO. 15)	Array element D
[0103]	TYR_{4-9}:	AVLYCL	(SEQ ID NO. 16)	Array element E

[0104] The six arrays had the following arrangements of elements after starting with an initiator methionine:

[0105]	pVAX-PC-A:	B-A-D-D-A-B-A-A
[0106]	pVAX-PC-B:	D-A-B-A-A-D-B-A
[0107]	pVAX-PC-C:	E-A-D-B-A-B-E-A-A
[0108]	pVAX-BC-A:	B-A-C-B-A-A-C-A
[0109]	pVAX-BC-B:	C-A-B-C-A-A-B-A

[0110] pVAX-BC-C: E-A-A-B-C-B-A-A

[0111] These arrays were inserted into the same vector backbone described in the examples above. The plasmid vectors were used to immunize mice essentially as described in Example 2 and the resulting CTL were tested for their ability to specifically lyse target cells pulsed with the peptide NY-ESO-1 157-165, corresponding to element B above. Both pVAX-PC-A and pVAX-BC-A were found to induce specific lytic activity. Comparing the contexts of the epitope (element B) in the various arrays, and particularly between pVAX-PC-A and pVAX-BC-A, between pVAX-PC-A and pVAX-PC-B, and between pVAX-BC-A and pVAX-BC-C, it was concluded that it was the first occurrence of the epitope in pVAX-PC-A and pVAX-BC-A that was being correctly processed and presented. In other words an initiator methionine followed by elements B-A constitute a substrate/liberation sequence for the presentation of element B. On this basis a new expression cassette for use as a vaccine was constructed encoding the following elements:

[0112] An initiator methionine,

[0113] NY-ESO-$1_{157\text{-}165}$ (**bold**) – a housekeeping epitope,

[0114] $SSX2_{41\text{-}49}$ (*italic*) – providing appropriate context for processing, and

[0115] NY-ESO-$1_{77\text{-}180}$ – to avoid "short sequence" problems and provide immune epitopes.

[0116] Thus the construct encodes the amino acid sequence:

[0117] M-**SLLMWITQC**-*KASEKIFYV*-
RCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTVSGNILTIRLTA
ADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQRR (SEQ ID NO. 17) and MSLLMWITQCKASEKIFYV (SEQ ID NO. 18) constitutes the liberation or substrate sequence. A polynucleotide encoding SEQ ID NO. 17 (SEQ ID NO. 19: nucleotides 12-380) was inserted into the same plasmid backbone as used for pMA2M generating the plasmid pN157.

Example 4

[0118] A construct similar to pN157 containing the whole epitope array from pVAX-PC-A was also made and designated pBPL. Thus the encoded amino acid sequence in pBPL is:

[0119] M-**SLLMWITQC**-*KASEKIFYV*-GLPSIPVHPI-GLPSIPVHPI-*KASEKIFYV*-
SLLMWITQC-*KASEKIFYV-KASEKIFYV*-
RCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTVSGNILTIRLTA
ADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQRR (SEQ ID NO. 20).

[0120] SEQ ID NO. 21 is the polynucleotide encoding SEQ ID NO. 20 used in pBPL.

[0121] A portion of SEQ ID NO. 20, *IKASEKIFYV*SLLMWITQC*KASEKIFYV*K (SEQ ID NO. 22) was made as a synthetic peptide and subjected to *in vitro* proteasomal digestion analysis with human immunoproteasome, utilizing both mass spectrometry and N-terminal pool sequencing. The identification of a cleavage after the C residue indicates that this segment of the construct can function as a substrate or liberation sequence for NY-ESO-$1_{157-165}$ (SEQ ID NO. 12) epitope (see Figure 6). Figure 7 shows the differential processing of the SLLMWITQC epitope (SEQ ID NO. 12) in its native context where the cleavage following the C is more efficiently produced by housekeeping than immunoproteasome. The immunoproteasome also produces a major cleavage internal to the epitope, between the T and the Q when the epitope is in its native context, but not in the context of SEQ ID NO. 22 (compare fig. 6 and 7).

Example 5

[0122] Screening of further epitope arrays led to the identification of constructs promoting the expression of the epitope SSX-2_{41-49} (SEQ ID NO. 13). In addition to some of the array elements defined in Example 3, the following additional elements were also used:

[0123] SSX-4_{57-65}:VMTKLGFKV (SEQ ID NO. 23) Array element F.

[0124] $PSMA_{730-739}$: RQIYVAAFTV (SEQ ID NO. 24) Array element G.

[0125] A construct, denoted CTLA02, encoding an initiator methionine and the array F-A-G-D-C-F-G-A, was found to successfully immunize HLA-A2 transgenic mice to generate a CTL response recognizing the peptide SSX-2_{41-49} (SEQ ID NO. 13).

[0126] As described above, it can be desirable to combine a sequence with substrate or liberation sequence function with one that can be processed into immune epitopes. Thus SSX-2_{15-183} (SEQ ID NO. 25) was combined with all or part of the array as follows:

[0127] CTLS1: F-A-G-D-C-F-G-A- SSX-2_{15-183} (SEQ ID NO. 26)

[0128] CTLS2: SSX-2_{15-183} - F-A-G-D-C-F-G-A (SEQ ID NO. 27)

[0129] CTLS3: F-A-G-D- SSX-2_{15-183} (SEQ ID NO. 28)

[0130] CTLS4: SSX-2_{15-183} -C-F-G-A (SEQ ID NO. 29).

[0131] All of the constructs except CTLS3 were able to induce CTL recognizing the peptide SSX-2_{41-49} (SEQ ID NO. 13). CTLS3 was the only one of these four constructs which did not include the second element A from CTLA02 suggesting that it was this second occurrence of the

element that provided substrate or liberation sequence function. In CTLS2 and CTLS4 the A element is at the C-terminal end of the array, as in CTLA02. In CTLS1 the A element is immediately followed by the SSX-2_{15-183} segment which begins with an alanine, a residue often found after proteasomal cleavage sites (Toes, R.E.M., et al., *J. Exp. Med.* 194:1-12, 2001). SEQ ID NO. 30 is the polynucleotide sequence encoding SEQ ID NO. 26 used in CTLS1, also called pCBP.

[0132] A portion of CTLS1 (SEQ ID NO. 26), encompassing array elements F-A-SSX-2_{15-23} with the sequence RQIYVAAFTV-*KASEKIFYV*-AQIPEKIQK (SEQ ID NO. 31), was made as a synthetic peptide and subjected to *in vitro* proteasomal digestion analysis with human immunoproteasome, utilizing both mass spectrometry and N-terminal pool sequencing. The observation that the C-terminus of the SSX-2_{41-49} epitope (SEQ ID NO. 13) was generated (see Figure 8) provided further evidence in support of substrate or liberation sequence function. The data in Figure 9 showed the differential processing of the SSX-2_{41-49} epitope, *KASEKIFYV* (SEQ ID NO. 13), in its native context, where the cleavage following the V was the predominant cleavage produced by housekeeping proteasome, while the immunoproteasome had several major cleavage sites elsewhere in the sequence. By moving this epitope into the context provided by SEQ ID NO. 31 the desired cleavage became a major one and its relative frequency compared to other immunoproteasome cleavages was increased (compare figs. 8 and 9). The data in figure 8B also showed the similarity in specificity of mouse and human immunoproteasome lending support to the usefulness of the transgenic mouse model to predict human antigen processing.

Example 6

[0133] Screening also revealed substrate or liberation sequence function for a tyrosinase epitope, $Tyr_{207-215}$ (SEQ ID NO. 32), as part of an array consisting of the sequence $[Tyr_{1-17}$- $Tyr_{207-215}]_4$, $[$MLLAVLYCLLWSFQTSA-FLPWHRLFL$]_4$, (SEQ ID NO. 33). The same vector backbone described above was used to express this array. This array differs from those of the other examples in that the Tyr_{1-17} segment, which was included as a source of immune epitopes, is used as a repeated element of the array. This is in contrast with the pattern shown in the other examples where sequence included as a source of immune epitopes and/or length occurred a single time at the beginning or end of the array, the remainder of which was made up of individual epitopes or shorter sequences.

Plasmid construction

[0134] The polynucleotide encoding SEQ ID NO. 33 was generated by assembly of annealed synthetic oligonucleotides. Four pairs of complementary oligonucleotides were synthesized which span the entire coding sequence with cohesive ends of the restriction sites of Afl II and EcoR I at either terminus. Each complementary pair of oligonucleotides were first annealed, the resultant DNA fragments were ligated stepwise, and the assembled DNA fragment was inserted into the same vector backbone described above pre-digested with Afl II/EcoR I. The construct was called CTLT2/pMEL and SEQ ID NO. 34 is the polynucleotide sequence used to encode SEQ ID NO. 33.

Example 7

Administration of a DNA plasmid formulation of a immunotherapeutic for melanoma to humans.

[0135] An MA2M melanoma vaccine with a sequence as described in Example 1 above, was formulated in 1% Benzyl alcohol, 1% ethyl alcohol, 0.5mM EDTA, citrate-phosphate, pH 7.6. Aliquots of 200, 400, and 600 μg DNA/ml were prepared for loading into MINIMED 407C infusion pumps. The catheter of a SILHOUETTE infusion set was placed into an inguinal lymph node visualized by ultrasound imaging. The pump and infusion set assembly was originally designed for the delivery of insulin to diabetics. The usual 17mm catheter was substituted with a 31mm catheter for this application. The infusion set was kept patent for 4 days (approximately 96 hours) with an infusion rate of about 25 μl/hour resulting in a total infused volume of approximately 2.4 ml. Thus the total administered dose per infusion was approximately 500, and 1000 μg; and can be 1500 μg, respectively, for the three concentrations described above. Following an infusion, subjects were given a 10 day rest period before starting a subsequent infusion. Given the continued residency of plasmid DNA in the lymph node after administration and the usual kinetics of CTL response following disappearance of antigen, this schedule will be sufficient to maintain the immunologic CTL response.

Example 8

[0136] SEQ ID NO. 22 is made as a synthetic peptide and packaged with a cationic lipid protein transfer reagent. The composition is infused directly into the inguinal lymph node (see example 7) at a rate of 200 to 600 μg of peptide per day for seven days, followed by seven days rest. An initial treatment of 3-8 cycles are conducted.

Example 9

[0137] A fusion protein is made by adding SEQ ID NO. 34 to the 3' end of a nucleotide sequence encoding herpes simplex virus 1 VP22 (SEQ ID NO. 42) in an appropriate mammalian expression vector; the vector used above is suitable. The vector is used to transform HEK 293 cells and 48 to 72 hours later the cells are pelleted, lysed and a soluble extract prepared. The fusion protein is purified by affinity chromatagraphy using an anti-VP22 monoclonal antibody. The purified fusion protein is administered intranodally at a rate of 10 to 100 μg per day for seven days, followed by seven days rest. An initial treatment of 3-8 cycles are conducted.

[0138] Further, the present invention can utilize various aspects of the following: U.S. Patent Application Nos. 09/380,534, filed on September 1, 1999, entitled A METHOD OF INDUCING A CTL RESPONSE; 09/776,232, filed on February 2, 2001, entitled METHOD OF INDUCING A CTL RESPONSE; 09/715,835, filed on November 16, 2000, entitled AVOIDANCE OF UNDESIRABLE REPLICATION INTERMEDIATES IN PLASMID PROPOGATION; 09/999,186, filed on November 7, 2001, entitled METHODS OF COMMERCIALIZING AN ANTIGEN; and Provisional U.S. Patent Application No 60/274,063, filed on March 7, 2001, entitled ANTI-NEOVASCULAR VACCINES FOR CANCER.

Table 11

Partial listing of SEQ ID NOS.

1	ELAGIGILTV	melan-A 26-35 (A27L)
2	Melan –A protein	Accession number: NP_005502
3	Tyrosinase protein	Accession number: P14679
4	MLLAVLYCLELAGIGILTVYMDGTMSQVGILT VILGVLLLIGCWYCRRRNGYRALMDKSLHVG TQCALTRRCPQEGFDHRDSKVSLQEKNCEPV	pMA2M expression product
5	MLLAVLYCLELAGIGILTVYMDGTMSQV	Liberation or substrate sequence for SEQ ID NO. 1 from pMA2M
6	MLLAVLYCL	tyrosinase 1-9
7	YMDGTMSQV	tyrosinase 369-377
8	EAAGIGILTV	melan-A 26-35
9	cttaagccaccatgttactagctgttttgtactgcctggaact agcagggatcggcatattgacagtgtatatgga tggaacaatgtcccaggtaggaattctgacagtgatcctggga gtcttactgctcatcggctgttggtattgtaga agacgaaatggatacagagccttgatggataaaagtcttcatg ttggcactcaatgtgccttaacaagaagatgcc cacaagaagggtttgatcatcgggacagcaaagtgtctcttca agagaaaaactgtgaacctgtgtagtgagcggc cgc	pMA2M insert
10	MVLYCLELAGIGILTVYMDGTAVLYCLELAGI	Epitope array from pVAXM2 and

	GILTVYMDGTMLAVLYCLELAGIGILTVYMD GTMSLLAVLYCLELAGIGILTV	pVAXM1
11	NY-ESO-1 protein	Accession number: P78358
12	SLLMWITQC	NY-ESO-1 157-165
13	KASEKIFYV	SSX-2 41-49
14	TQCFLPVFL	NY-ESO-1 163-171
15	GLPSIPVHPI	PSMA 288-297
16	AVLYCL	tyrosinase 4-9
17	MSLLMWITQCKASEKIFYVRCGARGPESRLLE FYLAMPFATPMEAELARRSLAQDAPPLPVPGV LLKEFTVSGNILTIRLTAADHRQLQLSISSCLQ QLSLLMWITQCFLPVFLAQPPSGQRR	pN157 expression product
18	MSLLMWITQCKASEKIFYV	liberation or substrate sequence for SEQ ID NO. 12 from pN157
19	cttaagccaccatgtccctgttgatgtggatcacgcagtgcaa agcttcggagaaaatcttctacgtacggtgcgg tgccaggggccggagagccgcctgcttgagttctacctcgcc atgcctttcgcgacacccatggaagcagagctg gcccgcaggagcctggcccaggatgccccaccgcttcccgtgc caggggtgcttctgaaggagttcactgtgtccg gcaacatactgactatccgactgactgctgcagaccaccgcca actgcagctctccatcagctcctgtctccagca gctttccctgttgatgtggatcacgcagtgctttctgcccgtg tttttggctcagcctccctcagggcagaggcgc tagtgagaattc	Insert for pN157
20	MSLLMWITQCKASEKIFYVGLPSIPVHPIGLPSI PVHPIKASEKIFYVSLLMWITQCKASEKIFYVK ASEKIFYVRCGARGPESRLLEFYLAMPFATPM EAELARRSLAQDAPPLPVPGVLLKEFTVSGNIL TIRLTAADHRQLQLSISSCLQQLSLLMWITQCF LPVFLAQPPSGQRR	pBPL expression product
21	atgtccctgttgatgtggatcacgcagtgcaaagcttcggaga aaatcttctatgtgggtcttccaagtattcctg ttcatccaattggtcttccaagtattcctgttcatccaattaa agcttcggagaaaatcttctatgtgtccctgtt gatgtggatcacgcagtgcaaagcttcggagaaaatcttctat gtgaaagcttcggagaaaatcttctacgtacgg tgcggtgccaggggccggagagccgcctgcttgagttctacc tcgccatgcctttcgcgacacccatggaagcag agctggcccgcaggagcctggcccaggatgccccaccgcttcc cgtgccaggggtgcttctgaaggagttcactgt gtccggcaacatactgactatccgactgactgctgcagaccac cgccaactgcagctctccatcagctcctgtctc cagcagctttccctgttgatgtggatcacgcagtgctttctgc ccgtgtttttggctcagcctccctcagggcaga ggcgctagtga	pBPL insert coding region
22	IKASEKIFYVSLLMWITQCKASEKIFYVK	Substrate in Fig. 6
23	VMTKLGFKV	$SSX\text{-}4_{57\text{-}65}$
24	RQIYVAAFTV	$PSMA_{730\text{-}739}$
25	AQIPEKIQKAFDDIAKYFSKEEWEKMKASEKIF YVYMKRKYEAMTKLGFKATLPPFMCNKRAE DFQGNDLDNDPNRGNQVERPQMTFGRLQGIS PKIMPKKPAEEGNDSEEVPEASGPQNDGKELC PPGKPTTSEKIHERSGPKRGEHAWTHRLRERK QLVIYEEISDP	$SSX\text{-}2_{15\text{-}183}$

26	MVMTKLGFKVKASEKIFYVRQIYVAAFTV GLPSIPVHPITQCFLPVFLVMTKLGFKVRQIYV AAFTVKASEKIFYVAQIPEKIQKAFDDIAKYFS KEEWEKMKASEKIFYVYMKRKYEAMTKLGF KATLPPFMCNKRAEDFQGNDLDNDPNRGNQ VERPQMTFGRLQGISPKIMPKKPAEEGNDSEE VPEASGPQNDGKELCPPGKPTTSEKIHERSGPK RGEHAWTHRLRERKQLVIYEEISDP	CTLS1/pCBP expression product
27	MAQIPEKIQKAFDDIAKYFSKEEWEKMKASE KIFYVYMKRKYEAMTKLGFKATLPPFMCNKR AEDFQGNDLDNDPNRGNQVERPQMTFGRLQ GISPKIMPKKPAEEGNDSEEVPEASGPQNDGK ELCPPGKPTTSEKIHERSGPKRGEHAWTHRLR ERKQLVIYEEISDPVMTKLGFKVKASEKIFYV RQIYVAAFTVGLPSIPVHPITQCFLPVFLVMTK LGFKVRQIYVAAFTVKASEKIFYV	CTLS2 expression product
28	MVMTKLGFKVKASEKIFYVRQIYVAAFTV GLPSIPVHPIAQIPEKIQKAFDDIAKYFSKEEWE KMKASEKIFYVYMKRKYEAMTKLGFKATLPP FMCNKRAEDFQGNDLDNDPNRGNQVERPQM TFGRLQGISPKIMPKKPAEEGNDSEEVPEASGP QNDGKELCPPGKPTTSEKIHERSGPKRGEHAW THRLRERKQLVIYEEISDP	CTLS3 expression product
29	MAQIPEKIQKAFDDIAKYFSKEEWEKMKASE KIFYVYMKRKYEAMTKLGFKATLPPFMCNKR AEDFQGNDLDNDPNRGNQVERPQMTFGRLQ GISPKIMPKKPAEEGNDSEEVPEASGPQNDGK ELCPPGKPTTSEKIHERSGPKRGEHAWTHRLR ERKQLVIYEEISDPTQCFLPVFLVMTKLGFKVR QIYVAAFTVKASEKIFYV	CTLS4 expression product
30	atggtcatgactaaactaggtttcaaggtcaaagcttcggaga aaatcttctatgtgagacagatttatgttgcag ccttcacagtgggtcttccaagtattcctgttcatccaattac gcagtgctttctgcccgtgtttttggtcatgac taaactaggtttcaaggtcagacagatttatgttgcagccttc acagtgaaagcttcggagaaaatcttctacgta gctcaaataccagagaagatccaaaaggccttcgatgatattg ccaaatacttctctaaggaagagtgggaaaaga tgaaagcctcggagaaaatcttctatgtgtatatgaagagaaa gtatgaggctatgactaaactaggtttcaaggc caccctcccacctttcatgtgtaataaacgggccgaagacttc caggggaatgatttggataatgaccctaaccgt gggaatcaggttgaacgtcctcagatgactttcggcaggctcc agggaatctccccgaagatcatgcccaagaagc cagcagaggaaggaaatgattcggaggaagtgccagaagcatc tggcccacaaaatgatgggaaagagctgtgccc cccgggaaaaccaactacctctgagaagattcacgagagatct ggacccaaaaggggggaacatgcctggacccac agactgcgtgagagaaaacagctggtgatttatgaagagatca gcgacccttagtga	pCBP insert coding region
31	RQIYVAAFTVKASEKIFYVAQIPEKIQK	Fig. 8 substrate/ CTLS1-2
32	FLPWHRLFL	$TYR_{207-215}$
33	MLLAVLYCLLWSFQTSAFLPWHRLFLMLLAV LYCLLWSFQTSAFLPWHRLFLMLLAVLYCLL	CTLT2/pMEL expression product

	WSFQTSAFLPWHRLFLMLLAVLYCLLWSFQT SAFLPWHRLFL	
34	atgctcctggctgttttgtactgcctgctgtggagtttccaga cctccgcttttctgccttggcatagactcttct tgatgctcctggctgttttgtactgcctgctgtggagtttcca gacctccgcttttctgccttggcatagactctt cttgatgctcctggctgttttgtactgcctgctgtggagtttc cagacctccgcttttctgccttggcatagactc ttcttgatgctcctggctgttttgtactgcctgctgtggagtt tccagacctccgcttttctgccttggcatagac tcttcttgtagtga	CTLT2/pMEL insert coding region
35	MELAN-A cDNA	Accession number: NM_005511
36	Tyrosinase cDNA	Accession number: NM_000372
37	NY-ESO-1 cDNA	Accession number: U87459
38	PSMA protein	Accession number: NP_004467
39	PSMA cDNA	Accession number: NM_004476
40	SSX-2 protein	Accession number: NP_003138
41	SSX-2 cDNA	Accession number: NM_003147
42	atgacctctcgccgctccgtgaagtcgggtccgcgggaggttccgcgc gatgagtacgaggatctgtactacaccccgtcttcaggtatggcgagtcc cgatagtccgcctgacacctcccgccgtggcgccctacagacacgctc gcgccagaggggcgaggtccgtttcgtccagtacgacgagtcggattat gccctctacgggggctcgtcatccgaagacgacgaacacccggaggt ccccccggacgcggcgtcccgtttccggggcggttttgtccggcccggg gcctgcgcgggcgcctccgccacccgctgggtccggaggggccgga cgcacacccaccaccgccccccgggcccccccgaacccagcgggtgg cgactaaggcccccgcggccccggcggcggagaccacccgcggca ggaaatcggcccagccagaatccgccgcactcccagacgcccccgcg tcgacggcgccaacccgatccaagacacccgcgcaggggctggcca gaaagctgcactttagcaccgcccccccaaaccccgacgcgccatgga ccccccgggtggccggctttaacaagcgcgtcttctgcgccgcggtcg ggcgcctggcggccatgcatgcccggatggcggcggtccagctctgg gacatgtcgcgtccgcgcacagacgaagacctcaacgaactccttggc atcaccaccatccgcgtgacggtctgcgagggcaaaaacctgcttcag cgcgccaacgagttggtgaatccagacgtggtgcaggacgtcgacgcg gccacggcgactcgagggcgttctgcggcgtcgcgccccaccgagcg acctcgagccccagcccgctccgcttctcgccccagacggcccgtcga g	From accession number: D10879 Herpes Simplex virus 1 UL49 coding sequence (VP22)
43	MTSRRSVKSGPREVPRDEYEDLYYTPSSGMAS PDSPPDTSRRGALFTQTRSRQRGEVRFVQYDE SDYALYGGSSSEDDEHPEVPRTRRPVSGAVLS GPGPARAPPPFTPAGSGGAGRTPTTAPRAPRT QRVATKAPAAPAAETTRGRKSAQPESAALPD APASTAPTFTRSKTPAQGLARKLHFSTAPPNP DAPWTPRVAGFNKRVFCAAVGRLAAMHARM AAVQLWDFTMSRPRTDEDLNELLGITTIRVTV CEGKNLLQRANELVNPDVVQDVDAATATRG RSAASRFTPTERPRAPARSASRPRRPVE	Accession number: P10233 Herpes Simplex virus 1 UL49/VP22 protein sequence

Melan-A mRNA sequence

LOCUS NM_005511 1524 bp mRNA PRI 14-OCT-2001

DEFINITION Homo sapiens melan-A (MLANA), mRNA.

ACCESSION NM_005511

VERSION NM_005511.1 GI:5031912

(SEQ ID NO. 2)

/translation="MPREDAHFIYGYPKKGHGHSYTTAEEAAGIGILTVILGVLLLIGCWYCRRRNGY
RALMDKSLHVGTQCALTRRCPQEGFDHRDSKVSLQEKNCEPVVPNAPPAYEKLSAEQSPPP
YSP"

(SEQ ID NO. 35)

ORIGIN

1 agcagacaga ggactctcat taaggaaggt gtcctgtgcc ctgaccctac aagatgccaa
61 gagaagatgc tcacttcatc tatggttacc ccaagaaggg gcacggccac tcttacacca
121 cggctgaaga ggccgctggg atcggcatcc tgacagtgat cctgggagtc ttactgctca
181 tcggctgttg gtattgtaga agacgaaatg gatacagagc cttgatggat aaaagtcttc
241 atgttggcac tcaatgtgcc ttaacaagaa gatgcccaca agaagggttt gatcatcggg
301 acagcaaagt gtctcttcaa gagaaaaact gtgaacctgt ggttcccaat gctccacctg
361 cttatgagaa actctctgca gaacagtcac caccacctta ttcaccttaa gagccagcga
421 gacacctgag acatgctgaa attatttctc tcacactttt gcttgaattt aatacagaca
481 tctaatgttc tcctttggaa tggtgtagga aaaatgcaag ccatctctaa taataagtca
541 gtgttaaaat tttagtaggt ccgctagcag tactaatcat gtgaggaaat gatgagaaat
601 attaaattgg gaaaactcca tcaataaatg ttgcaatgca tgatactatc tgtgccagag
661 gtaatgttag taaatccatg gtgttatttt ctgagagaca gaattcaagt gggtattctg
721 gggccatcca atttctcttt acttgaaatt tggctaataa caaactagtc aggttttcga
781 accttgaccg acatgaactg tacacagaat tgttccagta ctatggagtg ctcacaaagg
841 atacttttac aggttaagac aaagggttga ctggcctatt tatctgatca agaacatgtc
901 agcaatgtct ctttgtgctc taaaattcta ttatactaca ataatatatt gtaaagatcc
961 tatagctctt ttttttgag atggagtttc gcttttgttg cccaggctgg agtgcaatgg
1021 cgcgatcttg gctcaccata acctccgcct cccaggttca agcaattctc ctgccttagc
1081 ctcctgagta gctgggatta caggcgtgcg ccactatgcc tgactaattt tgtagtttta
1141 gtagagacgg ggtttctcca tgttggtcag gctggtctca aactcctgac ctcaggtgat

1201 ctgcccgcct cagcctccca aagtgctgga attacaggcg tgagccacca cgcctggctg
1261 gatcctatat cttaggtaag acatataacg cagtctaatt acatttcact tcaaggctca
1321 atgctattct aactaatgac aagtattttc tactaaacca gaaattggta gaaggattta
1381 aataagtaaa agctactatg tactgcctta gtgctgatgc ctgtgtactg ccttaaatgt
1441 acctatggca atttagctct cttgggttcc caaatccctc tcacaagaat gtgcagaaga
1501 aatcataaag gatcagagat tctg

Tyrosinase mRNA sequence

LOCUS NM_000372 1964 bp mRNA PRI 31-OCT-2000
DEFINITION Homo sapiens tyrosinase (oculocutaneous albinism IA) (TYR), mRNA.
ACCESSION NM_000372
VERSION NM_000372.1 GI:4507752

(SEQ ID NO. 3)
/translation="MLLAVLYCLLWSFQTSAGHFPRACVSSKNLMEKECCPPWSGDRS
PCGQLSGRGSCQNILLSNAPLGPQFPFTGVDDRESWPSVFYNRTCQCSGNFMGFNCGNCKFG
FWGPNCTERRLLVRRNIFDLSAPEKDKFFAYLTLAKHTISSDYVIPIGTYGQMKNGSTPMFND
INIYDLFVWMHYYVSMDALLGGSEIWRDIDFAHEAPAFLPWHRLFLLRWEQEIQKLTGDENF
TIPYWDWRDAEKCDICTDEYMGGQHPTNPNLLSPASFFSSWQIVCSRLEEYNSHQSLCNGTP
EGPLRRNPGNHDKSRTPRLPSSADVEFCLSLTQYESGSMDKAANFSFRNTLEGFASPLTGIAD
ASQSSMHNALHIYMNGTMSQVQGSANDPIFLLHHAFVDSIFEQWLRRHRPLQEVYPEANAPI
GHNRESYMVPFIPLYRNGDFFISSKDLGYDYSYLQDSDPDSFQDYIKSYLEQASRIWSWLLGA
AMVGAVLTALLAGLVSLLCRHKRKQLP EEKQPLLMEKEDYHSLYQSHL"

(SEQ ID NO. 36)
ORIGIN
1 atcactgtag tagtagctgg aaagagaaat ctgtgactcc aattagccag ttcctgcaga
61 ccttgtgagg actagaggaa gaatgctcct ggctgttttg tactgcctgc tgtggagttt
121 ccagacctcc gctggccatt tccctagagc ctgtgtctcc tctaagaacc tgatggagaa
181 ggaatgctgt ccaccgtgga gcggggacag gagtccctgt ggccagcttt caggcagagg
241 ttcctgtcag aatatccttc tgtccaatgc accacttggg cctcaatttc ccttcacagg
301 ggtggatgac cgggagtcgt ggccttccgt cttttataat aggacctgcc agtgctctgg

361 caacttcatg ggattcaact gtggaaactg caagtttggc ttttggggac caaactgcac
421 agagagacga ctcttggtga gaagaaacat cttcgatttg agtgccccag agaaggacaa
481 atttttgcc tacctcactt tagcaaagca taccatcagc tcagactatg tcatccccat
541 agggacctat ggccaaatga aaaatggatc aacacccatg tttaacgaca tcaatattta
601 tgacctcttt gtctggatgc attattatgt gtcaatggat gcactgcttg ggggatctga
661 aatctggaga gacattgatt ttgcccatga agcaccagct tttctgcctt ggcatagact
721 cttcttgttg cggtgggaac aagaaatcca gaagctgaca ggagatgaaa acttcactat
781 tccatattgg gactggcggg atgcagaaaa gtgtgacatt tgcacagatg agtacatggg
841 aggtcagcac cccacaaatc ctaacttact cagcccagca tcattcttct cctcttggca
901 gattgtctgt agccgattgg aggagtacaa cagccatcag tctttatgca atggaacgcc
961 cgagggacct ttacggcgta atcctggaaa ccatgacaaa tccagaaccc caaggctccc
1021 ctcttcagct gatgtagaat tttgcctgag tttgacccaa tatgaatctg gttccatgga
1081 taaagctgcc aatttcagct ttagaaatac actggaagga tttgctagtc cacttactgg
1141 gatagcggat gcctctcaaa gcagcatgca caatgccttg cacatctata tgaatggaac
1201 aatgtcccag gtacagggat ctgccaacga tcctatcttc cttcttcacc atgcatttgt
1261 tgacagtatt tttgagcagt ggctccgaag gcaccgtcct cttcaagaag tttatccaga
1321 agccaatgca cccattggac ataaccggga atcctacatg gttcctttta taccactgta
1381 cagaaatggt gatttcttta tttcatccaa agatctgggc tatgactata gctatctaca
1441 agattcagac ccagactctt ttcaagacta cattaagtcc tatttggaac aagcgagtcg
1501 gatctggtca tggctccttg gggcggcgat ggtaggggcc gtcctcactg ccctgctggc
1561 agggcttgtg agcttgctgt gtcgtcacaa gagaaagcag cttcctgaag aaaagcagcc
1621 actcctcatg gagaaagagg attaccacag cttgtatcag agccatttat aaaaggctta
1681 ggcaatagag tagggccaaa aagcctgacc tcactctaac tcaaagtaat gtccaggttc
1741 ccagagaata tctgctggta tttttctgta aagaccattt gcaaaattgt aacctaatac
1801 aaagtgtagc cttcttccaa ctcaggtaga acacacctgt ctttgtcttg ctgttttcac
1861 tcagcccttt taacattttc ccctaagccc atatgtctaa ggaaaggatg ctatttggta
1921 atgaggaact gttatttgta tgtgaattaa agtgctctta tttt

NY-ESO-1 mRNA sequence

LOCUS HSU87459 752 bp mRNA PRI 22-DEC-1999
DEFINITION Human autoimmunogenic cancer/testis antigen NY-ESO-1 mRNA, complete

cds.
ACCESSION U87459
VERSION U87459.1 GI:1890098

(SEQ ID NO. 11)

/translation="MQAEGRGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAAR
ASGPGGGAPRGPHGGAASGLNGCCRCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDA
PPLPVPGVLLKEFTVSGNILTIRLTAADHRQLQLSISSCLQQLSLLM
WITQCFLPVFLAQPPSGQRR"

(SEQ ID NO. 37)

ORIGIN

1 atcctcgtgg gccctgacct tctctctgag agccgggcag aggctccgga gccatgcagg
61 ccgaaggccg gggcacaggg ggttcgacgg gcgatgctga tggcccagga ggccctggca
121 ttcctgatgg cccaggggge aatgctggcg gcccaggaga ggcgggtgcc acgggcggca
181 gaggtccccg gggcgcaggg gcagcaaggg cctcggggcc gggaggaggc gccccgcggg
241 gtccgcatgg cggcgcggct tcagggctga atggatgctg cagatgcggg gccaggggge
301 cggagagccg cctgcttgag ttctacctcg ccatgccttt cgcgacaccc atggaagcag
361 agctggcccg caggagcctg gcccaggatg ccccaccgct tcccgtgcca ggggtgcttc
421 tgaaggagtt cactgtgtcc ggcaacatac tgactatccg actgactgct gcagaccacc
481 gccaactgca gctctccatc agctcctgtc tccagcagct ttccctgttg atgtggatca
541 cgcagtgctt tctgcccgtg tttttggctc agcctccctc agggcagagg cgctaagccc
601 agcctggcgc cccttcctag gtcatgcctc ctcccctagg gaatggtccc agcacgagtg
661 gccagttcat tgtgggggcc tgattgtttg tcgctggagg aggacggctt acatgtttgt
721 ttctgtagaa aataaaactg agctacgaaa aa

PSMA cDNA sequence

LOCUS NM_004476 2653 bp mRNA PRI 01-NOV-2000
DEFINITION Homo sapiens folate hydrolase (prostate-specific membrane antigen)
1 (FOLH1), mRNA.
ACCESSION NM_004476

VERSION NM_004476.1 GI:4758397

(SEQ ID NO. 38)

/translation="MWNLLHETDSAVATARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNIT
PKHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFGLDSVELAHYD
VLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSPQGMPEGDLVYVNY
ARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKNAQLAGAKGVILYSDPADYFAPGVK
SYPDGWNLPGGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYYDA
QKLLEKMGGSAPPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRG
AVEPDRYVILGGHRDSWVFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEF
GLLGSTEWAEENSRLLQERGVAYINADSSIEGNYTLRVDCTPLMYSLVHNLTKELKSPDEGF
EGKSLYESWTKKSPSPEFSGMPRISKLGSGNDFEVFFQRLGIASGRARYTKNWETNKFSGYPL
YHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSIVLPFDCRDYAVVLRKYADKIY
SISMKHPQEMKTYSVSFDSLFSAVKNFTEIASKFSERLQDFDKSNPIVLRMMNDQLMFLERAF
IDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYDALFDIESKVDPSKAWGEVKRQIYVAAFT
VQAAAETLSEVA"

(SEQ ID NO. 39)

ORIGIN

1 ctcaaaaggg gccggatttc cttctcctgg aggcagatgt tgcctctctc tctcgctcgg
61 attggttcag tgcactctag aaacactgct gtggtggaga aactggaccc caggtctgga
121 gcgaattcca gcctgcaggg ctgataagcg aggcattagt gagattgaga gagactttac
181 cccgccgtgg tggttggagg gcgcgcagta gagcagcagc acaggcgcgg gtcccgggag
241 gccggctctg ctcgcgccga gatgtggaat ctccttcacg aaaccgactc ggctgtggcc
301 accgcgcgcc gcccgcgctg gctgtgcgct ggggcgctgg tgctggcggg tggcttcttt
361 ctcctcggct tcctcttcgg gtggtttata aaatcctcca atgaagctac taacattact
421 ccaaagcata atatgaaagc atttttggat gaattgaaag ctgagaacat caagaagttc
481 ttatataatt ttacacagat accacattta gcaggaacag aacaaaactt tcagcttgca
541 aagcaaattc aatcccagtg gaaagaattt ggcctggatt ctgttgagct agcacattat
601 gatgtcctgt tgtcctaccc aaataagact catcccaact acatctcaat aattaatgaa
661 gatggaaatg agattttcaa cacatcatta tttgaaccac ctcctccagg atatgaaaat
721 gtttcggata ttgtaccacc tttcagtgct ttctctcctc aaggaatgcc agagggcgat
781 ctagtgtatg ttaactatgc acgaactgaa gacttcttta aattggaacg ggacatgaaa

841 atcaattgct ctgggaaaat tgtaattgcc agatatggga aagttttcag aggaaataag
901 gttaaaaatg cccagctggc aggggccaaa ggagtcattc tctactccga ccctgctgac
961 tactttgctc ctggggtgaa gtcctatcca gatggttgga atcttcctgg aggtggtgtc
1021 cagcgtggaa atatcctaaa tctgaatggt gcaggagacc ctctcacacc aggttaccca
1081 gcaaatgaat atgcttatag gcgtggaatt gcagaggctg ttggtcttcc aagtattcct
1141 gttcatccaa ttggatacta tgatgcacag aagctcctag aaaaaatggg tggctcagca
1201 ccaccagata gcagctggag aggaagtctc aaagtgccct acaatgttgg acctggcttt
1261 actggaaact tttctacaca aaaagtcaag atgcacatcc actctaccaa tgaagtgaca
1321 agaatttaca atgtgatagg tactctcaga ggagcagtgg aaccagacag atatgtcatt
1381 ctgggaggtc accgggactc atgggtgttt ggtggtattg accctcagag tggagcagct
1441 gttgttcatg aaattgtgag gagctttgga acactgaaaa aggaagggtg gagacctaga
1501 agaacaattt tgtttgcaag ctgggatgca gaagaatttg gtcttcttgg ttctactgag
1561 tgggcagagg agaattcaag actccttcaa gagcgtggcg tggcttatat taatgctgac
1621 tcatctatag aaggaaacta cactctgaga gttgattgta caccgctgat gtacagcttg
1681 gtacacaacc taacaaaaga gctgaaaagc cctgatgaag gctttgaagg caaatctctt
1741 tatgaaagtt ggactaaaaa aagtccttcc ccagagttca gtggcatgcc caggataagc
1801 aaattgggat ctggaaatga ttttgaggtg ttcttccaac gacttggaat tgcttcaggc
1861 agagcacggt atactaaaaa ttgggaaaca aacaaattca gcggctatcc actgtatcac
1921 agtgtctatg aaacatatga gttggtggaa aagttttatg atccaatgtt taaatatcac
1981 ctcactgtgg cccaggttcg aggagggatg gtgtttgagc tagccaattc catagtgctc
2041 ccttttgatt gtcgagatta tgctgtagtt ttaagaaagt atgctgacaa aatctacagt
2101 atttctatga aacatccaca ggaaatgaag acatacagtg tatcatttga ttcacttttt
2161 tctgcagtaa agaattttac agaaattgct tccaagttca gtgagagact ccaggacttt
2221 gacaaaagca acccaatagt attaagaatg atgaatgatc aactcatgtt tctggaaaga
2281 gcatttattg atccattagg gttaccagac aggccttttt ataggcatgt catctatgct
2341 ccaagcagcc acaacaagta tgcaggggag tcattcccag gaatttatga tgctctgttt
2401 gatattgaaa gcaaagtgga cccttccaag gcctggggag aagtgaagag acagatttat
2461 gttgcagcct tcacagtgca ggcagctgca gagactttga gtgaagtagc ctaagaggat
2521 tctttagaga atccgtattg aatttgtgtg gtatgtcact cagaaagaat cgtaatgggt
2581 atattgataa attttaaaat tggtatattt gaaataaagt tgaatattat atataaaaaa
2641 aaaaaaaaaa aaa

NM_003147 **Homo sapiens synovial sarcoma, X breakpoint 2 (SSX2), mRNA**

LOCUS NM_003147 766 bp mRNA PRI 14-MAR-2001
DEFINITION Homo sapiens synovial sarcoma, X breakpoint 2 (SSX2), mRNA.
ACCESSION NM_003147
VERSION NM_003147.1 GI:10337582

SEQ ID NO. 40

/translation="MNGDDAFARRPTVGAQIPEKIQKAFDDIAKYFSKEEWEKMKASE
KIFYVYMKRKYEAMTKLGFKATLPPFMCNKRAEDFQGNDLDNDPNRGNQVERPQMTFG
RLQGISPKIMPKKPAEEGNDSEEVPEASGPQNDGKELCPPGKPTTSEKIHERSGPKRG
EHAWTHRLRERKQLVIYEEISDPEEDDE"

SEQ ID NO 41

```
   1 ctctctttcg attcttccat actcagagta cgcacggtct gattttctct ttggattctt
  61 ccaaaatcag agtcagactg ctcccggtgc catgaacgga gacgacgcct ttgcaaggag
 121 acccacggtt ggtgctcaaa taccagagaa gatccaaaag gccttcgatg atattgccaa
 181 atacttctct aaggaagagt gggaaaagat gaaagcctcg gagaaaatct tctatgtgta
 241 tatgaagaga aagtatgagg ctatgactaa actaggtttc aaggccaccc tcccaccttt
 301 catgtgtaat aaacgggccg aagacttcca ggggaatgat ttggataatg accctaaccg
 361 tgggaatcag gttgaacgtc ctcagatgac tttcggcagg ctccagggaa tctccccgaa
 421 gatcatgccc aagaagccag cagaggaagg aaatgattcg gaggaagtgc cagaagcatc
 481 tggcccacaa aatgatggga aagagctgtg ccccccggga aaaccaacta cctctgagaa
 541 gattcacgag agatctggac ccaaaagggg ggaacatgcc tggacccaca gactgcgtga
 601 gagaaaacag ctggtgattt atgaagagat cagcgaccct gaggaagatg acgagtaact
 661 cccctcaggg atacgacaca tgcccatgat gagaagcaga acgtggtgac ctttcacgaa
 721 catgggcatg gctgcggacc cctcgtcatc aggtgcatag caagtg
```

WHAT IS CLAIMED IS:

1. A method of identifying a polypeptide suitable for epitope liberation, the method comprising the steps of:

identifying an epitope of interest;

providing a substrate polypeptide sequence comprising the epitope, wherein the substrate polypeptide permits processing by a proteasome;

contacting the substrate polypeptide with a composition comprising the proteasome, under conditions that support processing of the substrate polypeptide by the proteasome; and

assaying for liberation of the epitope.

2. The method of claim 1, wherein the epitope is embedded in the substrate polypeptide.

3. The method of claim 1, wherein the epitope is a housekeeping epitope.

4. The method of claim 1, wherein the substrate polypeptide is a synthetic peptide.

5. The method of claim 1, wherein the substrate polypeptide is a fusion protein.

6. The method of claim 1, wherein the contacting step comprises immunization with the substrate polypeptide.

7. The method of claim 1, wherein the substrate polypeptide is encoded by a polynucleotide.

8. The method of claim 7, wherein the contacting step comprises immunization with a vector comprising the polynucleotide.

9. The method of claim 7, wherein the contacting step comprises transforming a cell with a vector comprising the polynucleotide.

10. The method of claim 1, wherein the proteasome processing takes place *in vitro*.

11. The method of claim 1, wherein the assaying step consists of a technique selected from the group consisting of mass spectrometry, N-terminal pool sequencing, and HPLC.

12. The method of claim 1, wherein the assaying step comprises a T cell target recognition assay.

13. The method of claim 1, wherein the substrate polypeptide further comprises an array of additional epitopes.

14. The method of claim 13, wherein the array comprises a housekeeping and an immune epitope.

15. The method of claim 1, wherein the substrate polypeptide further comprises an array of epitopes and epitope clusters.

16. The method of Claim 1, wherein the proteasome is an immune proteasome.

17. A vector comprising a housekeeping epitope expression cassette, wherein the housekeeping epitope is derived from a target-associated antigen, and wherein the housekeeping epitope is liberatable from a translation product of the cassette by immunoproteasome processing.

18. The vector of claim 17, wherein the expression cassette encodes an array of two or more epitopes or at least one epitope and at least one epitope cluster.

19. The vector of claim 17, wherein the target-associated antigen is an antigen derived from or associated with a tumor or an intracellular parasite.

20. A method of activating a T cell comprising contacting the vector of claim 17 with an APC and contacting said APC with a T cell.

21. A substrate polypeptide comprising a housekeeping epitope wherein the housekeeping epitope can be liberated by immunoproteasome processing in a pAPC.

Figure 1. pMA2M

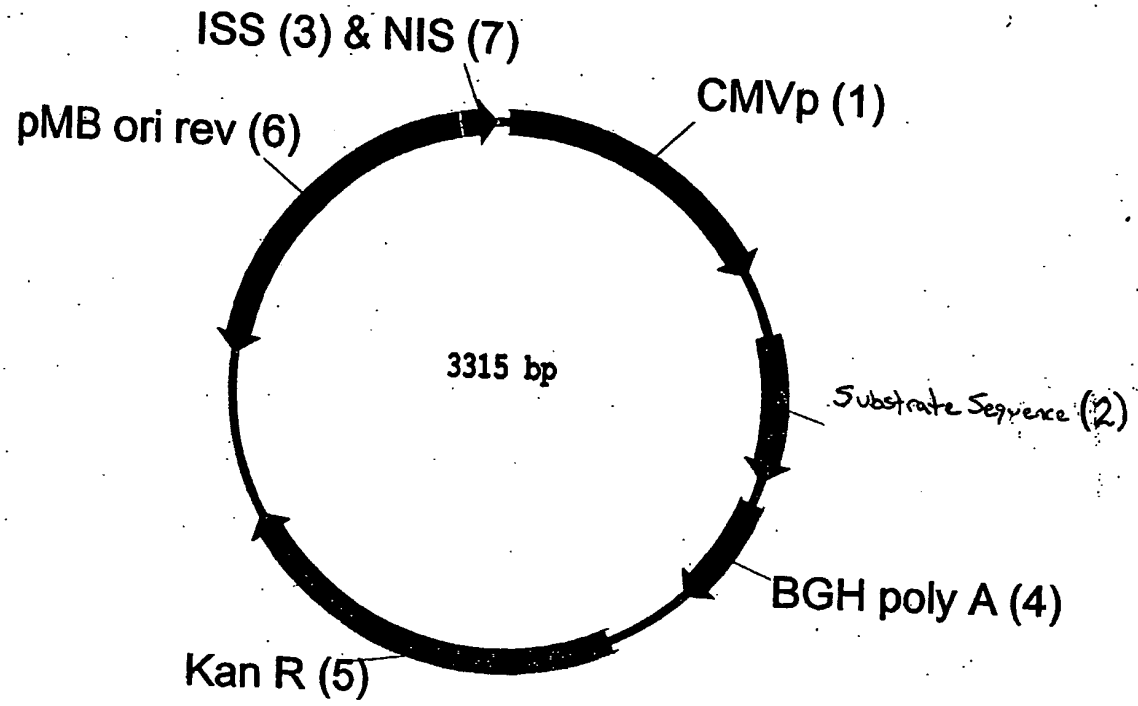


Figure Legend:

Code in Figure	Genetic Element	Region
~~1. CMVp~~	Cytomeglovirus Enhancer/Promotor	63-637
2. Substrate Sequence	Substrate Sequence containing epitope	696-983
3. ISS	Immunostimulatory Sequence	3220-3226
4. BGH poly A	Bovine Growth Hormone Polyadenylation Signal	1028-1045
5. Kan R	Kanamycin Resistance Gene	1431-2225
6. pMB ori rev	Bacterial pMB Origin of Replication	3165-2492
7. NIS	Nuclear Import sequence from Simian Virus 40-72bp repeat	3227-3304

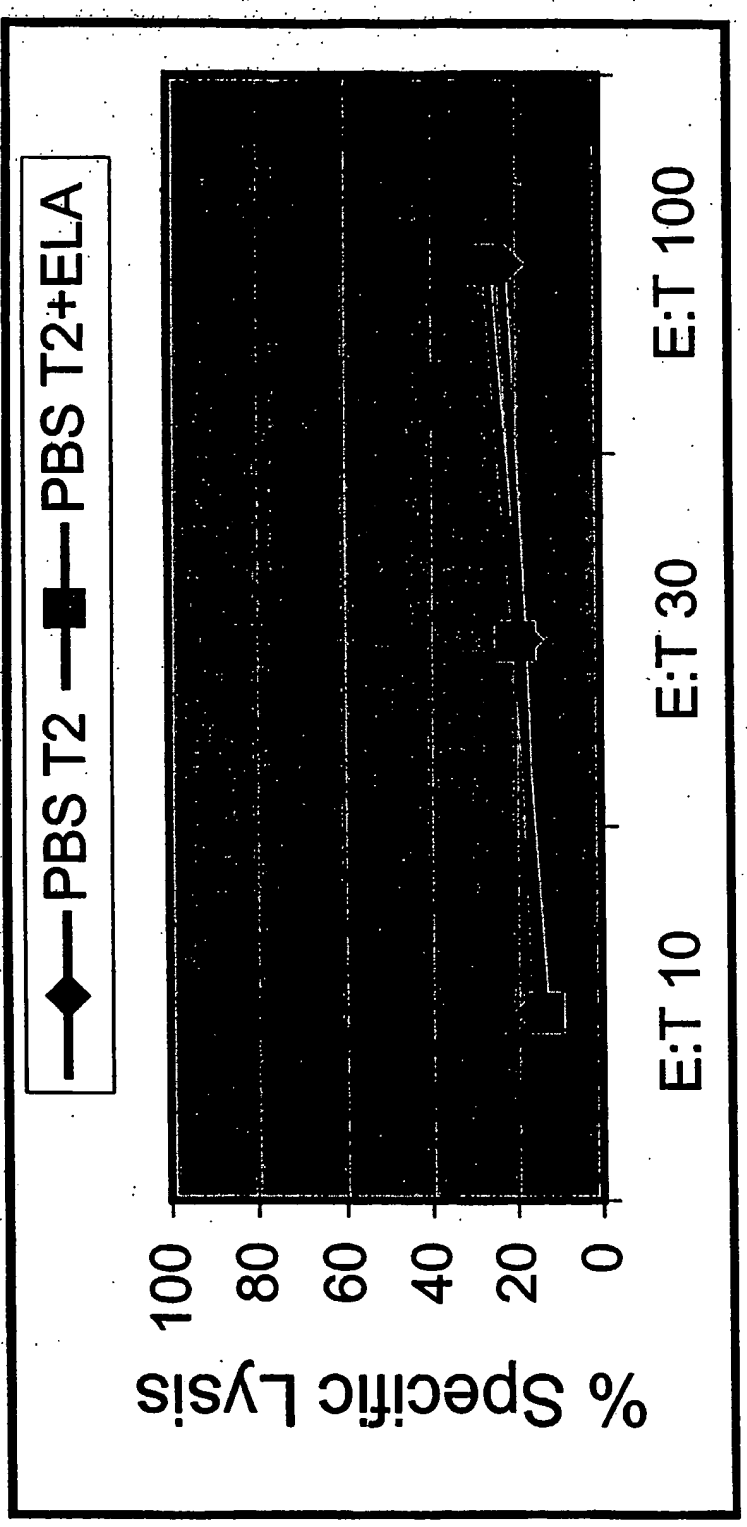


Figure 2. Lysis of ELAGIGILTV-pulsed and unpulsed T2 target cells by mock immunized CTL.

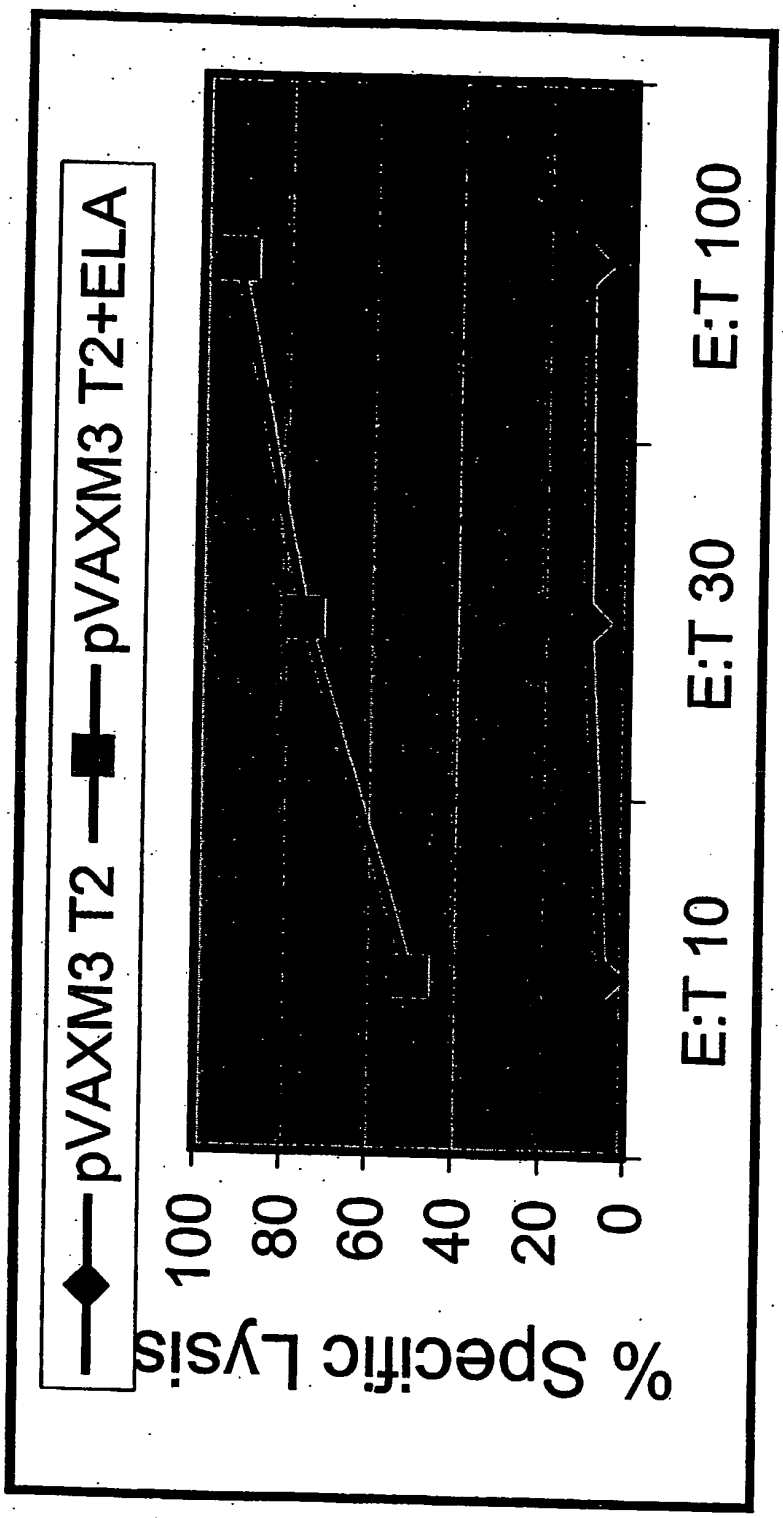


Figure 3. Lysis of ELAGIGILTV-pulsed and unpulsed T2 target cells by pVAXM3 immunized CTL.

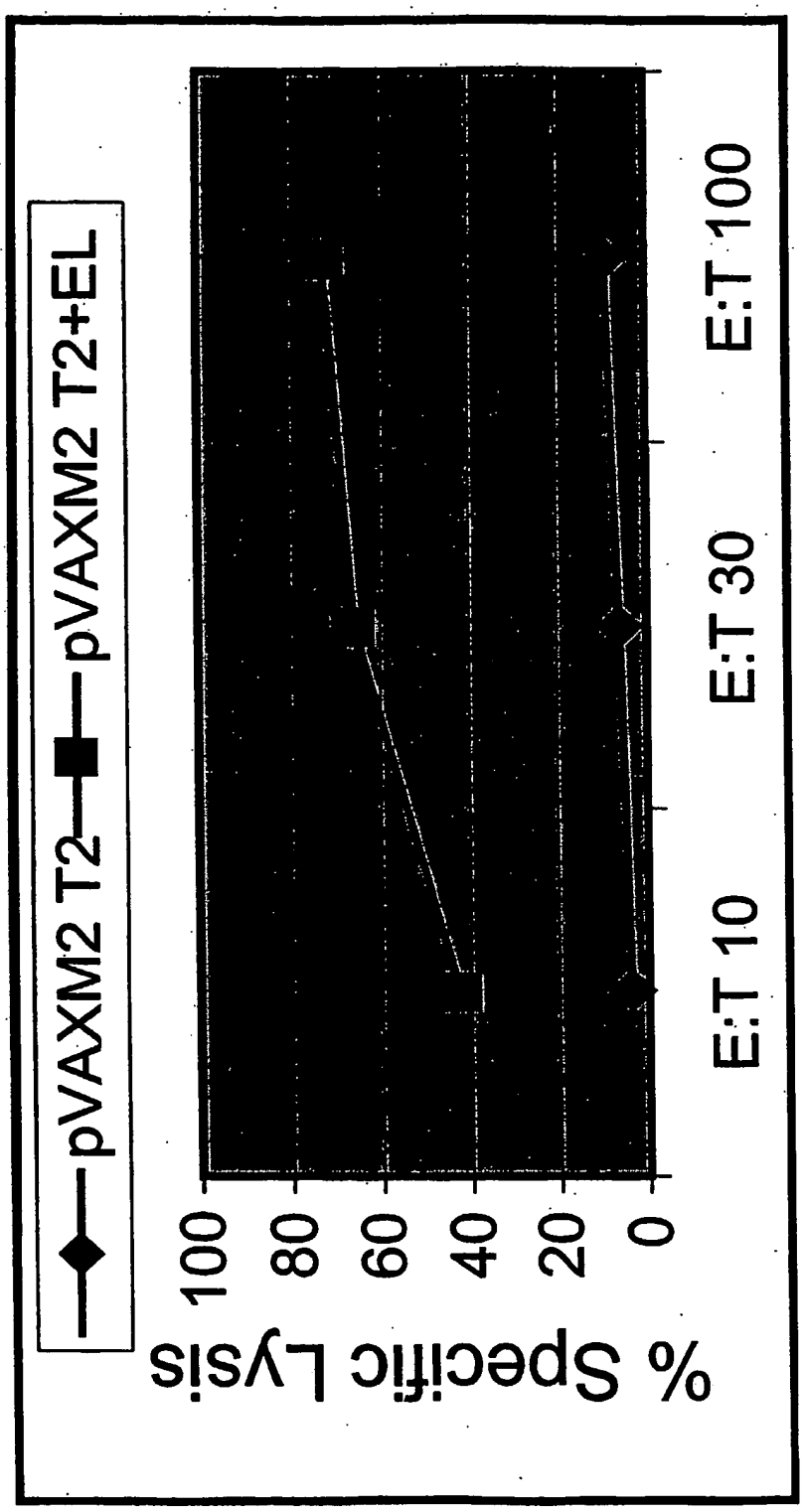


Figure 4. Lysis of ELAGIGILTV-pulsed and unpulsed T2 target cells by pVAXM2 immunized CTL.

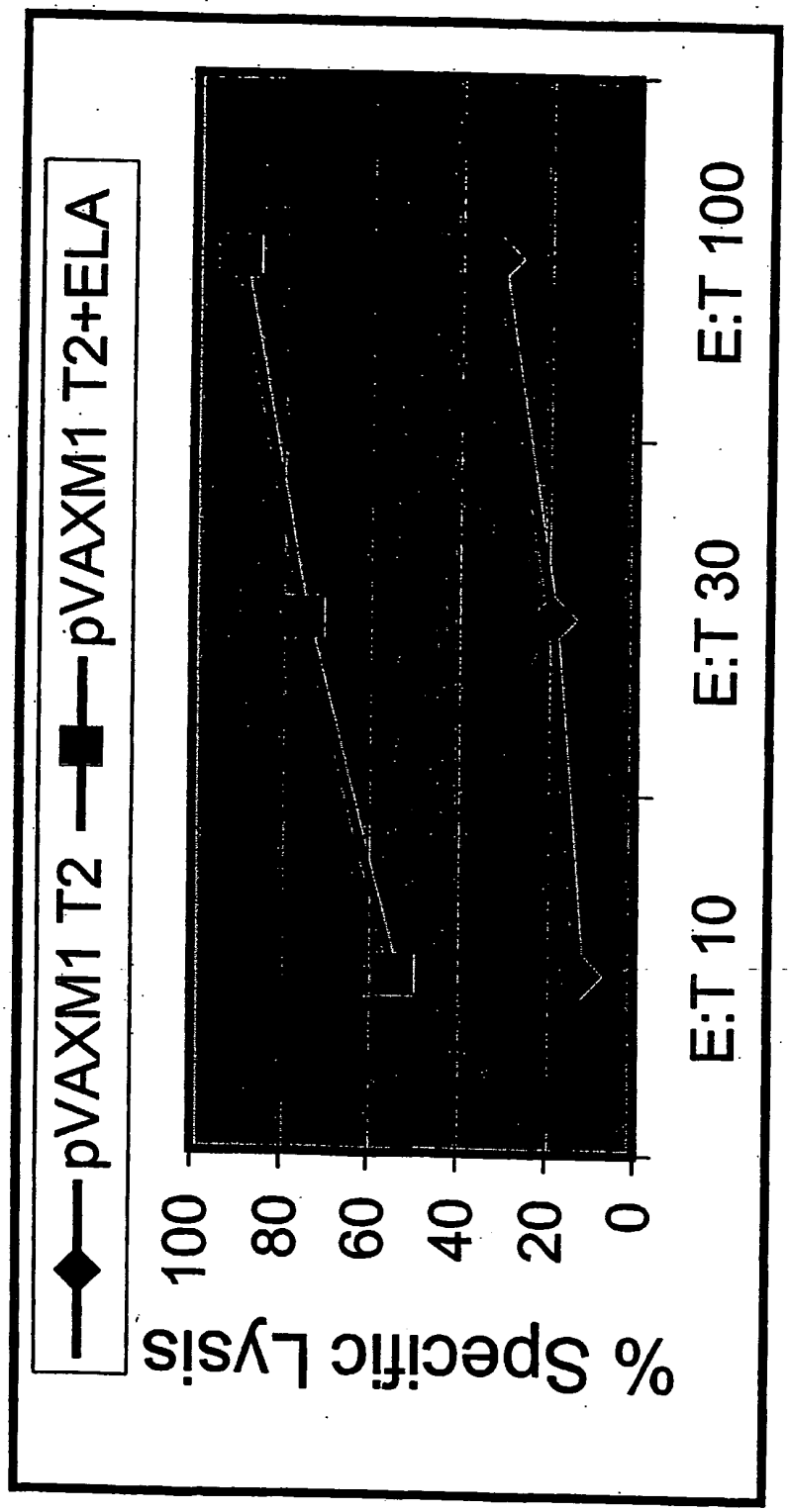


Figure 5. Lysis of ELAGIGILTV-pulsed and unpulsed T2 target cells by pVAXM1 immunized CTL.

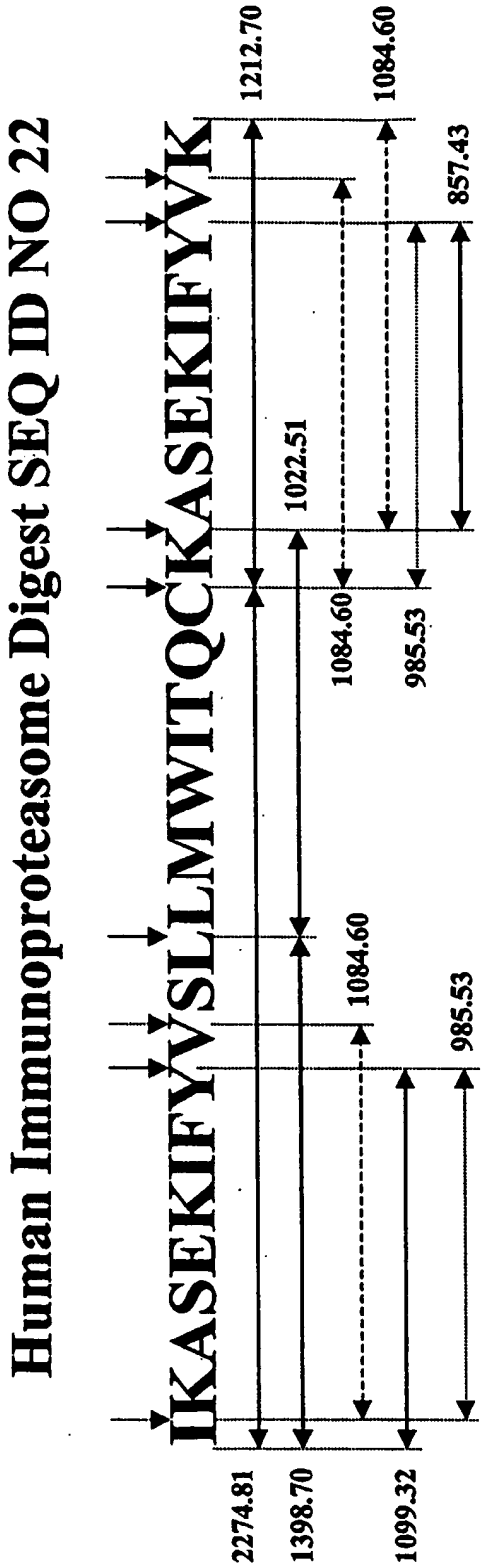
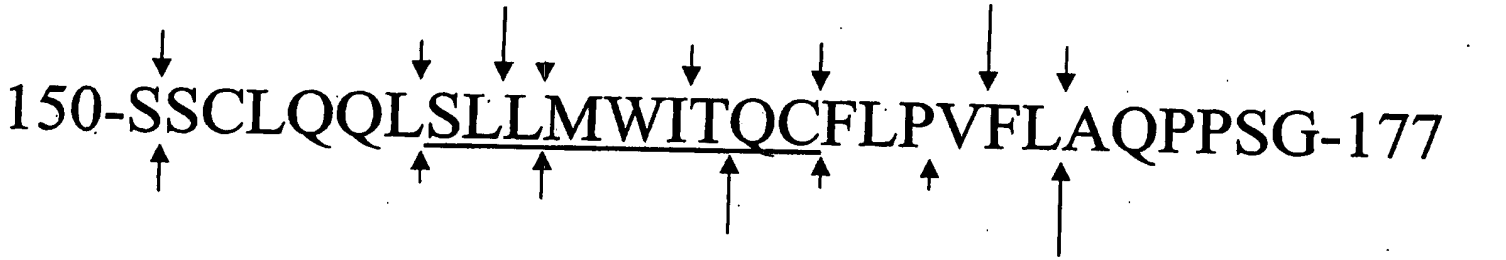

Human Immunoproteasome Digest SEQ ID NO 22
IKASEKIFYVSLLMWITQCKASEKIFYVK
1212.70
1084.60
857.43
1022.51
1084.60
985.53
1084.60
985.53
2274.81
1398.70
1099.32

Figure 6

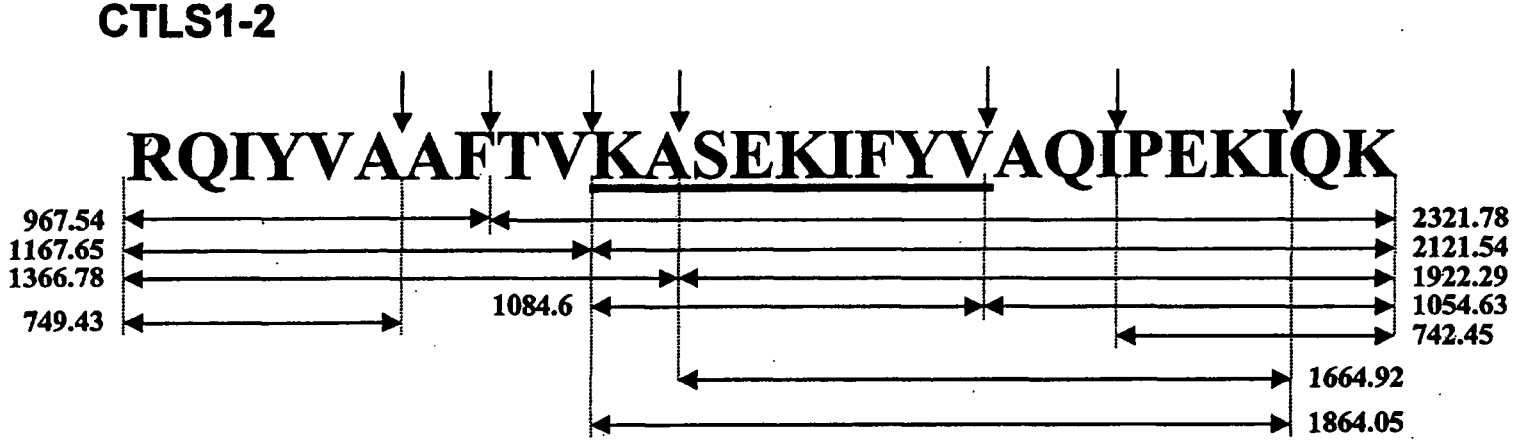
NY-ESO-1 150-177



Cleavage sites in the NY-ESO-1 150-177 substrate upon digestion with 20S housekeeping proteasome (upper arrows) and immunoproteasome (lower arrows). The size of each arrow indicates the efficiency of cleavage as determined by pool sequencing analysis. The epitope NY-ESO-1 157-165 (SEQ ID NO. 12) is underlined.

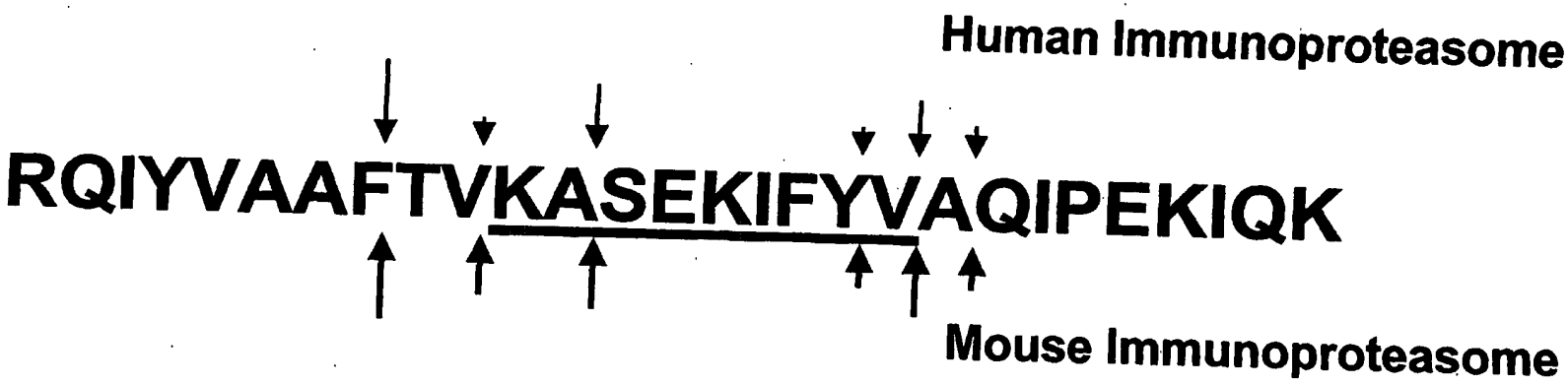
Figure 7

Human Immunoproteasome Digest of SEQ ID NO. 31



Cleavage sites in the CTLS1-2 substrate upon digestion with immunoproteasome (isolated from γ-IFN treated HeLa cells).. The sequence of epitope SSX2 41-49 (SEQ ID NO. 13) is underlined.

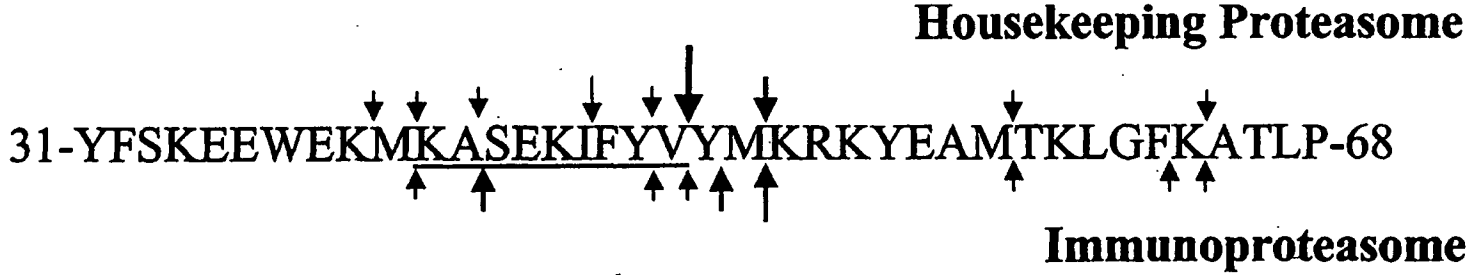
Figure 8A



Comparisons of CTLS1-2 substrate (SEQ ID NO. 31) digested by 20S human immunoproteasome versus mouse immunoproteasome. The size of each arrow indicates the efficiency of cleavage as determined by N-terminal pool sequencing analysis. The sequence of epitope SSX2 41-49 (SEQ ID NO. 13) is underlined.

Figure 8B

SSX2 31-68



Cleavage sites in the SSX2 31-68 substrate upon digestion with 20S housekeeping proteasome (isolated from erythrocytes) (upper arrows) and immunoproteasome (isolated from γ-IFN treated Hela cells) (lower arrows). The size of each arrow indicates the efficiency of cleavage as determined by N-terminal pool sequencing analysis. The epitope SSX2 41-49 (SEQ ID NO. 13) is underlined.

Figure 9